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NEWS 1		Web Page URLs for STN Seminar Schedule - N. America
NEWS 2	Apr 08	"Ask CAS" for self-help around the clock
NEWS 3	Apr 09	BEILSTEIN: Reload and Implementation of a New Subject Area
NEWS 4	Apr 09	ZDB will be removed from STN
NEWS 5	Apr 19	US Patent Applications available in IFICDB, IFIPAT, and IFIUDB
NEWS 6	Apr 22	Records from IP.com available in CAPLUS, HCAPLUS, and ZCAPLUS
NEWS 7	Apr 22	BIOSIS Gene Names now available in TOXCENTER
NEWS 8	Apr 22	Federal Research in Progress (FEDRIP) now available
NEWS 9	Jun 03	New e-mail delivery for search results now available
NEWS 10	Jun 10	MEDLINE Reload
NEWS 11	Jun 10	PCTFULL has been reloaded
NEWS 12	Jul 02	FOREGE no longer contains STANDARDS file segment
NEWS 13	Jul 22	USAN to be reloaded July 28, 2002; saved answer sets no longer valid
NEWS 14	Jul 29	Enhanced polymer searching in REGISTRY
NEWS 15	Jul 30	NETFIRST to be removed from STN
NEWS 16	Aug 08	CANCERLIT reload
NEWS 17	Aug 08	PHARMAMarketLetter(PHARMAML) - new on STN
NEWS 18	Aug 08	NTIS has been reloaded and enhanced
NEWS 19	Aug 19	Aquatic Toxicity Information Retrieval (AQUIRE) now available on STN
NEWS 20	Aug 19	IFIPAT, IFICDB, and IFIUDB have been reloaded
NEWS 21	Aug 19	The MEDLINE file segment of TOXCENTER has been reloaded
NEWS 22	Aug 26	Sequence searching in REGISTRY enhanced
NEWS 23	Sep 03	JAPIO has been reloaded and enhanced
NEWS 24	Sep 16	Experimental properties added to the REGISTRY file
NEWS 25	Sep 16	CA Section Thesaurus available in CAPLUS and CA
NEWS 26	Oct 01	CASREACT Enriched with Reactions from 1907 to 1985
NEWS 27	Oct 21	EVENTLINE has been reloaded
NEWS 28	Oct 24	BEILSTEIN adds new search fields
NEWS 29	Oct 24	Nutraceuticals International (NUTRACEUT) now available on STN
NEWS 30	Oct 25	MEDLINE SDI run of October 8, 2002
NEWS 31	Nov 18	DKILIT has been renamed APOLLIT
NEWS 32	Nov 25	More calculated properties added to REGISTRY
NEWS 33	Dec 02	TIBKAT will be removed from STN
NEWS 34	Dec 04	CSA files on STN
NEWS 35	Dec 17	PCTFULL now covers WP/PCT Applications from 1978 to date
NEWS 36	Dec 17	TOXCENTER enhanced with additional content
NEWS 37	Dec 17	Adis Clinical Trials Insight now available on STN
NEWS 38	Dec 30	ISMEC no longer available
NEWS 39	Jan 13	Indexing added to some pre-1967 records in CA/CAPLUS
NEWS 40	Jan 21	NUTRACEUT offering one free connect hour in February 2003
NEWS 41	Jan 21	PHARMAML offering one free connect hour in February 2003
NEWS 42	Jan 29	Simultaneous left and right truncation added to COMPENDEX, ENERGY, INSPEC
NEWS 43	Feb 13	CANCERLIT is no longer being updated
NEWS 44	Feb 24	METADEX enhancements
NEWS 45	Feb 24	PCTGEN now available on STN

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 NEWS 46  Feb 24  TEMA now available on STN
 NEWS 47  Feb 26  NTIS now allows simultaneous left and right truncation
 NEWS 48  Feb 26  PCTFULL now contains images
 NEWS 49  Mar 04  SDI PACKAGE for monthly delivery of multifile SDI results
 NEWS 50  Mar 19  APOLLIT offering free connect time in April 2003

 NEWS EXPRESS  January 6 CURRENT WINDOWS VERSION IS V6.01a,
               CURRENT MACINTOSH VERSION IS V6.0b(ENG) AND V6.0Jb(JP),
               AND CURRENT DISCOVER FILE IS DATED 01 OCTOBER 2002
 NEWS HOURS    STN Operating Hours Plus Help Desk Availability
 NEWS INTER    General Internet Information
 NEWS LOGIN    Welcome Banner and News Items
 NEWS PHONE    Direct Dial and Telecommunication Network Access to STN
 NEWS WWW      CAS World Wide Web Site (general information)

Enter NEWS followed by the item number or name to see news on that
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=> file .biotech
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                       0.21       0.21

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=> s dimention? (1) electrophoresis
MISSING OPERATOR 'IMENTION? (1'
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> s dimention? (n) electrophoresis
L1          16 DIMENTION? (N) ELECTROPHORESIS

=> s (two or 2) electrophoresis
MISSING OPERATOR 2) ELECTROPHOR
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.
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=> s (two or 2) and electrophoresis
L2      359717 (TWO OR 2) AND ELECTROPHORESIS

=> marker? or target?
MARKER? IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s marker? or target?
L3     1774220 MARKER? OR TARGET?

=> s l2 and l3
L4       22445 L2 AND L3

=> s l4 and disease
L5        3260 L4 AND DISEASE

=> s l5 and (obesity or osteoporosis or diabetes or osteoarthritis or hypertension)
L6         214 L5 AND (OBESITY OR OSTEOPOROSIS OR DIABETES OR OSTEOARTHRITIS
               OR HYPERTENSION)

=> dup rem
ENTER L# LIST OR (END):l6
PROCESSING COMPLETED FOR L6
L7          150 DUP REM L6 (64 DUPLICATES REMOVED)

=> d ti l7 1-50

L7   ANSWER 1 OF 150  EMBASE  COPYRIGHT 2003 ELSEVIER SCI. B.V.
TI   Protein carbonyl groups as biomarkers of oxidative stress.

L7    ANSWER 2 OF 150  BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI    Determining risk of developing cataracts for the manufacture of a
      medicament for treating cataracts, by evaluating a nucleic acid for the
      presence of Z-4 or Z-2 allele of the aldose reductase gene;
         DNA microarray and DNA probe for gene detection and disease
         diagnosis

L7    ANSWER 3 OF 150  BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI    New isolated human cation transporter 84226 polypeptides and nucleic acid
      molecules, for treating or preventing a disorder of aberrant activity of
      84226-expressing cell, e.g. pancreatic cancer or metal transport-related
      disorder;
         vector-mediated recombinant glutathione-S-transferase fusion protein
         gene transfer and expression in Escherichia coli for disease
         diagnosis and gene therapy

L7    ANSWER 4 OF 150  BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI    Developing gene panels for diagnosing or treating unwanted side effects
      of medicaments and diseases, e.g. cancers, comprises analyzing the
      expression and methylation status of specific genes;
         for use in central nervous system, disorder, psychotic disorder,
         cardiovascular disease, respiratory system disorder,
         gastrointestinal disorder, skin disease, muscular disorder,
         inflammation, infection, endocrine disorder, metabolic disease
         , leukemia and cancer diagnosis and therapy

L7    ANSWER 5 OF 150  BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI    New isolated polynucleotide sequences expressed in chondrocytes from a
      fetus, normal individual or patients with osteoarthritis useful
      as markers for constructing chondrocyte-specific microarrays
      for diagnosing osteoarthritis;
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recombinant protein production, expressed sequence tag and DNA array for pharmacogenomics

L7 ANSWER 6 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI New PRO842 polypeptides having structural homology to interleukin-8, useful for treating or diagnosing a mammal with an inflammatory **disease** or immune related **disease**, e.g. rheumatoid arthritis, **osteoarthritis** or allergic **disease**;
vector-mediated gene transfer and expression in host cell for recombinant protein production, drug screening and gene therapy

L7 ANSWER 7 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Herpes simplex virus-based amplicon vector has large capacity cloning vector, herpes virus origin of replication, cleavage/packaging signal and genomic DNA fragment, and infects/delivers genomic DNA to **target** cell;
virus vector expression in host cell, bacterium artificial chromosome and site-specific recombination use in **disease** therapy and gene therapy

L7 ANSWER 8 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Identifying single nucleotide polymorphisms at defined positions in **target** nucleic acids, by utilizing oligonucleotide primers that contain a part of an interrupted restriction endonuclease recognition sequence;
polymerase chain reaction and restriction enzyme for SNP detection and **disease** diagnosis

L7 ANSWER 9 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Novel schizophrenia-related voltage-gated ion channel polypeptide and polynucleotide useful for identifying modulators and for diagnosing, treating schizophrenia, bipolar disorder or central nervous system disorders;
vector-mediated recombinant CanIon protein gene transfer and expression in mammal host cell for cardiovascular **disease** diagnosis, prognosis and gene therapy

L7 ANSWER 10 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Novel osteoblast polypeptide, designated OBP1 and polynucleotide encoding it, useful for treating bone disorders e.g. **osteoporosis**, **osteoarthritis**, osteomalacia, Maroteaux-Lamy syndrome, and as diagnostic **markers**;
recombinant protein production and agonist and antagonist use in **disease** therapy and gene therapy

L7 ANSWER 11 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Novel Zyctor19 polypeptides and polynucleotides useful for stimulating immune responses in animals for producing antibodies, and for treating autoimmune diseases, leukemia and asthma;
recombinant protein, fusion protein and antibody for use in diagnosis and therapy and as a recombinant vaccine

L7 ANSWER 12 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Diagnosing **diabetes** or susceptibility to **diabetes** in individual by typing insulin receptor gene region or insulin receptor protein in sample obtained from individual;
insulin receptor protein region typing, and transgenic animal model use in **disease** therapy and diagnosis

L7 ANSWER 13 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Method, oligonucleotides and arrays for parallel measurement of genetic variations, based on the incorporation of unique restriction endonuclease restriction sites flanking and encompassing genetic variation loci;
DNA array, restriction endonuclease restriction site, constant

recognition sequence useful for mutation associated **disease** identification

L7 ANSWER 14 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Novel antisense oligonucleotide which inhibits expression of phosphorylase kinase beta, useful for treating metabolic disorder e.g. **diabetes**, prevent or delay infection, inflammation or tumor formation;
sense and antisense oligonucleotide and enzyme expression inhibition useful in **disease** gene therapy

L7 ANSWER 15 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Model system for modeling nitric oxide (NO)-mediated modulation of apoptosis in cells, involves identifying genes whose expression is modulated after modulating intracellular NO concentration in the cells;
HeLa cell gene and expressed sequence tag identification, and expression profiling

L7 ANSWER 16 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Polynucleotide sequences encoding human secretory proteins useful for gene therapy of e.g. genetic deficiency disorders, cancers, and diseases caused by intracellular parasites;
recombinant protein gene production via plasmid expression in host cell, sense, antisense, agonist, antagonist, transgenic animal, antibody, cell culture, DNA array and polymerase chain reaction useful in **disease** gene therapy and drug screening

L7 ANSWER 17 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Identifying specifically cleavable peptide, useful for **targeted** drug delivery and developing protease inhibitors, by incubating test compound with peptide-nucleic acid fusion;
metallo protease cleavage site identification and DNA library construction, useful for therapy, diagnosis and drug delivery

L7 ANSWER 18 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Novel antisense compound useful for treating type **2** **diabetes**, cancer and **obesity**, is **targeted** to nucleic acid encoding human protein phosphatase 1B, and hybridizes and inhibits PTP1B expression;
useful for gene therapy and functional genomics

L7 ANSWER 19 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Set of novel map-related biallelic **markers**, preferably located on **obesity** disorder-associated chromosomal regions on chromosomes 3, 10 and 19, useful, for e.g. detecting statistical correlations between **marker** allele and a phenotype;
DNA array, bioinformatic software and bioinformatic hardware for DNA analysis and pharmacogenomics

L7 ANSWER 20 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Producing a polyunsaturated fatty acid (PUFA), useful in dietary supplements and in treating diseases e.g., cancer, comprises expressing human delta 5-desaturase enzyme and exposing enzyme to substrate PUFA to convert to product PUFA;
recombinant enzyme protein production via plasmid expression in host cell for use in producing a polyunsaturated fatty acid for use in therapy

L7 ANSWER 21 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Novel expression vector comprising DNA segment encoding human zalpha33 polypeptide, operably linked to transcription promoter and terminator, useful for transforming host cells which are used to produce zalpha33 polypeptide;
vector-mediated gene transfer and expression in host cell for

recombinant protein production, drug screening and gene therapy

L7 ANSWER 22 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Detecting increased risk for developing inflammatory disorder in a mammal, involves detecting a copy of interleukin-1 beta gene haplotype in the mammal comprising cytosine nucleotides at specific positions;
risk assessment for developing inflammatory disorder associated with interleukin-1-beta copy number and pharmacogenetics

L7 ANSWER 23 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Novel isolated neurotransmitter-gated ion channel superfamily member, designated 14691 polypeptide, for treating angiogenesis, cardiovascular, endothelial cell, kidney, neurological, metabolic and immune disorders;
recombinant protein production and sense and antisense sequence use in gene therapy

L7 ANSWER 24 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI New ABCA12 polypeptide and nucleic acid, useful for manufacturing a medicament for preventing and/or treating diseases resulting from dysfunctions in lipophilic substance transport, e.g. lamellar ichthyosis, congenital cataract;
vector-mediated gene transfer, expression in host cell and antibody for recombinant protein production, drug screening and gene therapy

L7 ANSWER 25 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI New isolated stem cell growth factor-like polypeptide and polynucleotide, useful in therapeutic, diagnostic or research fields, e.g. inducing differentiation of embryonic and adult stem cells or in treating cancer or hemophilia;
vector-mediated gene transfer and expression in host cell for recombinant protein production, drug screening and gene therapy

L7 ANSWER 26 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Detecting if an organism is homozygous or heterozygous in a **target** sequence, by combining differential hybridization or restriction endonuclease digestion with immobilized array technology or electrophoretic separation;
gene mutation detection in plant, mammal or human using DNA primer and DNA probe for **disease** therapy and propagation

L7 ANSWER 27 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Use of genes and their expression profiles associated with osteoblast differentiation for screening modulators bone formation, for diagnosing or treating e.g. **osteoporosis**, or as **markers** for the differentiation process;
expression profiling and drug screening useful for **osteoporosis** gene therapy and diagnosis

L7 ANSWER 28 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI New non-genetic based protein **disease markers** for **obesity**, **osteoporosis**, **diabetes**, osteoathritis and **hypertension**, useful in diagnosis and monitoring of treatment for these diseases and to screen for therapeutic compounds;
two-dimensional **electrophoresis** and antisense oligonucleotide for protein distribution study, drug screening, proteomics analysis and potential gene therapy

L7 ANSWER 29 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI New antisense compound which is **targeted** to nucleic acid encoding phosphorylase kinase alpha 1 and inhibits expression of kinase protein, useful for treating a condition associated with kinase, e.g. **diabetes**;
phosphorylase-kinase-specific antisense oligonucleotide for use in

gene therapy of **diabetes**

L7 ANSWER 30 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Generating reference library of restriction fragments from pooled nucleic acids, by using reference population of restriction fragments to compare frequencies of polymorphic sequences between different population pools;
restriction fragment reference library generation, single stranded nuclease, and polymerase chain reaction for **disease** -associated gene identification and plant-associated phenotype trait screening

L7 ANSWER 31 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Novel isolated SIGLEC (sialic acid-binding Ig-related lectin) protein molecules useful for treating immune system diseases such as asthma, leukemia, allergic rhinitis, psoriasis, conjunctivitis, Crohn's **disease**;
vector-mediated recombinant gene transfer and expression in host cell for autoimmune, leukemia and asthma **disease** gene therapy, diagnosis and prognosis

L7 ANSWER 32 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Lipid-raft **targeted** derivative of a soluble polypeptide e.g. a soluble complement regulatory molecule for treating disorders involving complement activity and various inflammatory, neurological and immune disorders;
vector-mediated gene transfer and expression in host cell for recombinant protein production and **disease** therapy

L7 ANSWER 33 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI A polynucleotide encoding a TZON7 polypeptide or a biologically active fragment for the preparation of diagnostic and pharmaceutical compositions for use in e.g., organ transplantation and in the treatment of asthma;
vector-mediated recombinant protein gene transfer and expression in host cell, antibody, antisense and drug screening for **disease** and disorder diagnosis and gene therapy

L7 ANSWER 34 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI New human nucleic acid molecule, designated 61833, which encodes a novel pyridoxal-dependant decarboxylase, useful for treating cellular proliferative or differentiative disorders, cardiovascular disorders, and/or brain disorders;
plasmid vector-mediated recombinant protein gene transfer and expression in COS cell for **disease** diagnosis and gene therapy

L7 ANSWER 35 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Novel polypeptide useful for diagnosis, prognosis, prevention, and treatment of immune, hyperproliferative, renal, respiratory, cardiovascular, reproductive, endocrine, gastrointestinal and neurological disorders;
recombinant protein production via plasmid expression in host cell use in **disease** therapy and gene therapy

L7 ANSWER 36 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Novel respiratory system related polypeptide useful for diagnosis, prognosis, prevention, and treatment of immune, hyperproliferative, renal, respiratory, cardiovascular, reproductive or gastrointestinal disorders;
human recombinant protein production and antibody useful for gene therapy, diagnosis and prognosis

L7 ANSWER 37 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Novel polypeptide useful for diagnosis, prognosis, prevention, and

treatment of immune, hyperproliferative, renal, respiratory, cardiovascular, reproductive, endocrine, gastrointestinal and neurological disorders;
vector-mediated recombinant protein gene transfer and expression in host cell for use in gene therapy

L7 ANSWER 38 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Novel polypeptide useful for diagnosis, prognosis, prevention, and treatment of immune, hyperproliferative, renal, respiratory, cardiovascular, reproductive, endocrine, gastrointestinal and neurological disorders;
vector-mediated recombinant protein gene transfer and expression in host cell for use in **disease** diagnosis, prognosis, prevention, therapy and gene therapy

L7 ANSWER 39 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI New colorectal cancer polypeptide for diagnosing, prognosing, preventing, and treating immune, hyperproliferative, liver, kidney, reproductive disorders and for identifying modulators of therapeutic use;
recombinant protein production for use in therapy, gene therapy, recombinant vaccine and nucleic acid vaccine and for drug screening

L7 ANSWER 40 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Novel polypeptide useful for diagnosis, prognosis, prevention, and treatment of immune, hyperproliferative, renal, respiratory, cardiovascular, reproductive, endocrine, gastrointestinal and neurological disorders;
vector-mediated gene transfer and expression in host cell for recombinant protein production, drug screening and gene therapy

L7 ANSWER 41 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Novel DNA-binding protein useful for diagnosis, prognosis, prevention and treatment of immune, hyperproliferative, respiratory, cardiovascular, reproductive, endocrine, gastrointestinal and neurological disorders;
vector plasmid pQE-9-mediated recombinant protein gene transfer and expression in host cell for use in **disease** diagnosis, prognosis, prevention, therapy, gene therapy, recombinant vaccine and nucleic acid vaccine preparation

L7 ANSWER 42 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Novel PRO polypeptides and nucleic acids encoding the polypeptides, useful for preparing a medicament for the treatment of inflammatory and immune related disorders;
vector-mediated gene transfer, expression in host cell and antibody for recombinant protein production and drug screening

L7 ANSWER 43 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Novel secreted and transmembrane polypeptides and polynucleotides useful for diagnosis and treatment of inflammatory disorders and immune-related diseases, and identifying modulators;
vector-mediated recombinant protein gene transfer and expression in Chinese hamster ovary cell culture, Escherichia coli or yeast cell for use in drug screening and gene therapy

L7 ANSWER 44 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Making transgenic avian lacking expression of endogenous immunoglobulin by inactivating endogenous immunoglobulin heavy chain locus in a avian cell and generating an avian from the cell;
fowl, turkey, duck, goose or quail transgenic animal construction and monoclonal antibody for animal breeding

L7 ANSWER 45 OF 150 CAPLUS COPYRIGHT 2003 ACS
TI Ras-MEK-ERK1/**2** signaling pathway in the production of inflammatory and neuropathic pain and uses for analgesic screening

L7 ANSWER 46 OF 150 CAPLUS COPYRIGHT 2003 ACS
TI Mitochondrial protein **targets** for drug screening and therapeutic intervention identified using mass spectrometry

L7 ANSWER 47 OF 150 CAPLUS COPYRIGHT 2003 ACS
TI Method for the development of panels of genes for diagnosis and therapy based on the expression and methylation status of the genes

L7 ANSWER 48 OF 150 CAPLUS COPYRIGHT 2003 ACS
TI Method for the determination of at least one functional polymorphism in the nucleotide sequence of a preselected candidate gene and its applications

L7 ANSWER 49 OF 150 CAPLUS COPYRIGHT 2003 ACS
TI Non-genetic based protein **disease markers**

L7 ANSWER 50 OF 150 CAPLUS COPYRIGHT 2003 ACS
TI Non-genetic based protein **disease markers**

=> d ibib abs l7 1-50

L7 ANSWER 1 OF 150 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 2003072085 EMBASE
TITLE: Protein carbonyl groups as biomarkers of oxidative stress.
AUTHOR: Dalle-Donne I.; Rossi R.; Giustarini D.; Milzani A.; Colombo R.
CORPORATE SOURCE: I. Dalle-Donne, Department of Biology, University of Milan, via Celoria 26, I-20133 Milan, Italy. quack@mailserver.unimi.it
SOURCE: Clinica Chimica Acta, (2003) 329/1-2 (23-38). Refs: 108
ISSN: 0009-8981 CODEN: CCATAR
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 006 Internal Medicine
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Oxidative stress, an imbalance toward the pro-oxidant side of the pro-oxidant/antioxidant homeostasis, occurs in several human diseases. Among these diseases are those in which high levels of protein carbonyl (CO) groups have been observed, including Alzheimer's **disease** (AD), rheumatoid arthritis, **diabetes**, sepsis, chronic renal failure, and respiratory distress syndrome. What relationships might be among high level of protein CO groups, oxidative stress, and diseases remain uncertain. The usage of protein CO groups as biomarkers of oxidative stress has some advantages in comparison with the measurement of other oxidation products because of the relative early formation and the relative stability of carbonylated proteins. Most of the assays for detection of protein CO groups involve derivatisation of the carbonyl group with **2**,4-dinitrophenylhydrazine (DNPH), which leads to formation of a stable dinitrophenyl (DNP) hydrazone product. This then can be detected by various means, such as spectrophotometric assay, enzyme-linked immunosorbent assay (ELISA), and one-dimensional or **two**-dimensional **electrophoresis** followed by Western blot immunoassay. At present, the measurement of protein CO groups after their derivatisation with DNPH is the most widely utilized measure of protein oxidation.

L7 ANSWER 2 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2003-06558 BIOTECHDS
TITLE: Determining risk of developing cataracts for the manufacture

of a medicament for treating cataracts, by evaluating a nucleic acid for the presence of Z-4 or Z-**2** allele of the aldose reductase gene;
DNA microarray and DNA probe for gene detection and **disease** diagnosis

AUTHOR: CRITCHLEY J A J H; NG M C Y; LEE S C; COCKRAM C S; CHAN J C N
PATENT ASSIGNEE: UNIV CHINESE HONG KONG; WEST C P
PATENT INFO: WO 2002079506 10 Oct 2002
APPLICATION INFO: WO 2002-CN214 29 Mar 2002
PRIORITY INFO: US 2001-280529 30 Mar 2001; US 2001-280529 30 Mar 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-058436 [05]
AN 2003-06558 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Determining the risk of developing cataract in a mammal, comprises evaluating a sample comprising nucleic acid from the mammal for presence of a Z-4 allele and a Z-**2** allele of the aldose reductase gene (an absence of Z-4 allele and presence of Z-**2** allele indicates an increased risk for developing cataract in the mammal and where an absence of Z-**2** allele and presence of Z-4 allele indicates decreased risk for developing cataract in the mammal).

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a method for detecting increased or decreased risk of developing cataract in an individual of Chinese descent having non-insulin dependent **diabetes**; (**2**) a method for treating a mammal having an increased risk of developing cataract; (3) a primer pair comprising a first member having 19-bp sequence and a second member comprising 22-bp sequence; or (4) an array of nucleic acid probes comprising a nucleotide sequence of at least 10 nucleotide bases of 138-bp sequence that hybridize to the nucleic acid encoding the microsatellite **marker** of an aldose reductase gene.

BIOTECHNOLOGY - Preferred Method: Determining risk of developing cataract in a mammal further comprises contacting the Z-**2** or Z-4 allele with a sequencing microarray. The evaluating step is performed by contacting the sample with one or more nucleic acid probes, each of which comprises a sequence that hybridizes to the nucleotide region encoding a microsatellite **marker** of an aldose reductase gene. Preferably, it is performed by a technique consisting of probe hybridization, in situ hybridization, nuclease analysis, chromatography, autoradiography, fluorography, Southern blot analysis, direct sequencing, restriction enzyme fragment analysis, single-locus DNA profiling, multi-locus DNA profiling, microarray analysis, fragment electrophoretic mobility or mobility shift assay. Detecting increased or decreased risk of developing cataract in an individual of Chinese descent having non-insulin dependent **diabetes** comprises: (a) amplifying a Z-**2** or Z-4 allele of an aldose reductase gene in a nucleic acid sample from the individual using primers comprising 19- or 22-bp sequence, where amplified sequences are obtained; (b) hybridizing the amplified sequences under high stringency conditions with a probe comprising a detectable label and a sequence that hybridizes to the Z-**2** or Z-4 allele; and (c) screening for the detectable label, where the presence of the Z-**2** or Z-4 allele indicates increased risk of developing cataract and where the absence of the Z-**2** or Z-4 allele indicates decreased risk of developing cataract. The mammal has non-insulin dependent **diabetes**. It is a human of Chinese descent. Treating a mammal having an increased risk of developing cataract comprises providing the mammal with an inhibitor of aldose reductase. Preferred Primer: The primer is a polynucleotide primer for amplification of a microsatellite **marker** of an aldose reductase gene. Preferred Array: The array of nucleotide probes is a sequencing microarray and is arranged on a solid support in multiple discrete regions of distinct nucleic acid strands. The probe is radioactively or non-radioactively labeled. It comprises digoxigenin, enzyme, fluorescent,

nick-translation, photobiotin, PCR or an end label. Its nucleotide sequence comprises no more than 3350 bases. It consists of DNA, RNA or cDNA.

ACTIVITY - Ophthalmological. No biological data given.

MECHANISM OF ACTION - Aldose-Reductase-Inhibitor.

USE - The method is useful for treating a human having an increased risk of developing cataract (claimed).(37 pages)

L7 ANSWER 3 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2003-06751 BIOTECHDS
TITLE: New isolated human cation transporter 84226 polypeptides and nucleic acid molecules, for treating or preventing a disorder of aberrant activity of 84226-expressing cell, e.g. pancreatic cancer or metal transport-related disorder; vector-mediated recombinant glutathione-S-transferase fusion protein gene transfer and expression in Escherichia coli for **disease** diagnosis and gene therapy
AUTHOR: CURTIS R A J
PATENT ASSIGNEE: MILLENNIUM PHARM INC
PATENT INFO: WO 2002079427 10 Oct 2002
APPLICATION INFO: WO 2002-US9728 28 Mar 2002
PRIORITY INFO: US 2001-279281 28 Mar 2001; US 2001-279281 28 Mar 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-046802 [04]
AN 2003-06751 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - An isolated human cation transporter, designated as 84226 polypeptide comprising a 372 residue amino acid sequence (S2), given in the specification, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated 84226 nucleic acid molecule comprising: (a) a 1630 base pair sequence (S1), given in the specification; and (b) a nucleic acid molecule that encodes an 84226 polypeptide; (2) a host cell containing the 84226 nucleic acid molecule; (3) an antibody or its antigen-binding fragment that selectively binds to 84226 polypeptide; (4) a method for producing 84226 polypeptide; (5) detecting the presence of 84226 polypeptide comprising: (a) contacting the sample with an antibody that selectively binds to the polypeptide; and (b) determining if the compound binds to the polypeptide in the sample; (6) detecting the presence of 84226 nucleic acid molecule in a sample comprising: (a) contacting the sample with a nucleic acid probe or primer that selectively hybridizes to the nucleic acid; and (b) determining if the probe or primer binds to a nucleic acid in the sample; (7) kits comprising instructions for use, and: (a) a compound that selectively binds to the polypeptide in the sample; or (b) a nucleic acid probe or primer that selectively hybridizes to 84226 nucleic acid molecule; (8) identifying a compound that binds to 84226 polypeptide comprising: (a) contacting the polypeptide or a cell expressing the polypeptide with a test compound; and (b) determining if the polypeptide binds to the test compound; (9) modulating the activity of 84226 polypeptide comprising contacting the polypeptide or a cell expressing the polypeptide with a compound that binds to the polypeptide in a sufficient concentration to modulate the activity of the polypeptide; (10) inhibiting aberrant activity of an 84226-expressing cell comprising contacting the cell with a compound that modulates the activity or expression of 84226 polypeptide, in an amount effective to reduce or inhibit the aberrant activity of the cell; and (11) treating or preventing a disorder characterized by aberrant activity of an 84226-expressing cell in a subject by administering to the subject a compound that modulates the activity or expression of 84226 nucleic acid molecule, so that the aberrant activity of the 84226-expressing cell is reduced or inhibited.

BIOTECHNOLOGY - Preparation: Producing 84226 polypeptide comprises culturing the host cell of (1) under conditions in which the nucleic acid

molecule is expressed (claimed). Preferred Nucleic Acid: The nucleic acid molecule further comprises a vector nucleic acid sequence, or a nucleic acid sequence encoding a heterologous polypeptide. Preferred Polypeptide: The polypeptide further comprises heterologous amino acid sequences. Preferred Method: The method of (6), where the sample comprises mRNA molecules and is contacted with a nucleic acid probe. The compound used in the methods above is selected from the group of a peptide, a phosphopeptide, a small organic molecule, and an antibody. The 84226-expressing cell is a pancreas cell.

ACTIVITY - Cytostatic; Anorectic; Antidiabetic; Analgesic; Antilipemic; Antiinflammatory; Antiarteriosclerotic; Antiarrhythmic; Cardiant; Nephrotropic; Hypotensive. No biological data is given.

MECHANISM OF ACTION - Gene Therapy.

USE - The 84226 nucleic acid molecules and polypeptides are useful for diagnosing, treating or preventing a disorder characterized by aberrant activity of an 84226-expressing cell, such as conditions involving metal transport-related disorders, disorders associated with cellular toxicity resulting from aberrant or deficient cation diffusion, pancreatic disorders (e.g. pancreatic cancer or pancreatitis), metabolic disorders (e.g. **obesity**, anorexia nervosa, cachexia, lipid disorders or **diabetes**), pain disorders, or aberrant or deficient cellular proliferation or differentiation. The 84226 molecules are also useful as diagnostic **targets** and therapeutic agents for controlling heart disorder (e.g. **hypertension**, atherosclerosis, coronary artery **disease** or arrhythmias) or kidney disorders (e.g. amyloidosis, glomerulonephritis or Goodpasture's syndrome), or as pharmacogenomic **markers**. The methods are useful for evaluating the efficacy of a treatment of a disorder or the efficacy of a therapeutic or prophylactic agent. The polynucleotides can be used for chromosome mapping, tissue typing or in forensic biology.

ADMINISTRATION - Dosage is 0.001-30, preferably 5-6 mg/kg. Administration may be parenteral, e.g. intravenous, intradermal or subcutaneous, oral, e.g. inhalation, transdermal or topical, transmucosal, or rectal means.

EXAMPLE - 84226 was fused to glutathione-S-transferase (GST) and the fusion polypeptide was expressed in Escherichia coli strain PEB199. Expression of the glutathione-S-transferase (GST)-84226 fusion protein in PEB199 was induced with isopropyl-beta-D-thiogalactopyranose (IPTG). The recombinant fusion polypeptide was purified from crude bacterial lysates of the induced PEB199 strain by affinity chromatography on glutathione beads. Using polyacrylamide gel electrophoretic analysis of the polypeptide purified from the bacterial lysates, the molecular weight of the resultant fusion polypeptide was determined. (117 pages)

L7 ANSWER 4 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2003-03538 BIOTECHDS
TITLE: Developing gene panels for diagnosing or treating unwanted side effects of medicaments and diseases, e.g. cancers, comprises analyzing the expression and methylation status of specific genes;
for use in central nervous system, disorder, psychotic disorder, cardiovascular **disease**, respiratory system disorder, gastrointestinal disorder, skin **disease**, muscular disorder, inflammation, infection, endocrine disorder, metabolic **disease**, leukemia and cancer diagnosis and therapy
AUTHOR: OLEK A; BERLIN K
PATENT ASSIGNEE: EPIGENOMICS AG
PATENT INFO: WO 2002070742 12 Sep 2002
APPLICATION INFO: WO 2002-EP2255 1 Mar 2002
PRIORITY INFO: US 2001-272549 1 Mar 2001; US 2001-272549 1 Mar 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-698760 [75]

AN 2003-03538 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - Developing gene panels for diagnostic and therapeutic purposes comprising isolating a biological sample from a biological material, selecting the gene exhibiting a different expression level, analyzing the level of cytosine methylation in the methylation relevant regions of the selected gene, selecting the gene exhibiting a different level of cytosine methylation and adding selected genes from the second knowledge base to a gene panel, is new.

DETAILED DESCRIPTION - Developing gene panels for diagnostic and therapeutic purposes particularly comprising: (a) isolating at least one biological sample from each of at least **two** groups of biological material containing mRNA and/or proteins; (b) analyzing the expression level of at least one gene in at least one of the biological samples; (c) selecting the genes exhibiting a different expression level between the at least **two** groups of biological material, where a first knowledge base is generated; (d) analyzing the level of cytosine methylation in the methylation relevant regions of at least one gene of at least one of the biological samples, where the gene is selected on the basis of the first knowledge base; (e) selecting the genes exhibiting a different level of cytosine methylation between the at least **two** groups of biological material, where a second knowledge base is generated; and (f) adding selected genes from the second knowledge base to a gene panel. INDEPENDENT CLAIMS are also included for the following: (1) a gene panel obtained by the method of developing gene panels for diagnostic and therapeutic purposes; (**2**) a device for generating a gene panel comprising means for generating first and second knowledge bases and means for adding selected genes from the second knowledge base to a gene panel; or (3) a method for diagnosing or treating a **disease** or medical condition.

BIOTECHNOLOGY - Preferred Method: The steps in developing gene panels for diagnostic and therapeutic purposes are repeated for at least 100 times. The identical or different biological material, or their combination is used in step (a). The steps are performed in the following order: step (a), step (d), step (e), step (b), step (c) or step (f). The biological material is isolated by means of a biopsy, by means of an operation on an individual, by means of a dissection, derived from a preserved biological sample, collected from body fluids and/or collected directly from the environment. The biological material comprises a eukaryotic and/or prokaryotic cell line, a biopsy sample, blood, sputum, feces, urine, cerebral liquid, tissue embedded in paraffin, tissue derived from eyes, intestine, brain, heart, prostate, kidney, lung, breast or liver, and/or histological samples. At least one of the biological samples is derived from biological material of healthy and/or diseased individuals. The isolation of the biological sample comprises isolating subcellular compartments, organelles, macromolecular structures and multiprotein complexes, partial or complete preparation of the mRNA, reverse transcription or partial digestion of the material with an enzyme selected from proteases, RNAses and/or DNAses. Diagnosing a **disease** comprising: (a) providing the gene panel; (b) analyzing the level of cytosine methylation at selected sites of the DNA based on the gene panel in biological material of at least one diseased individual with a known **disease** or medical condition and/or at least one healthy individual, for generating a first knowledge base; (c) analyzing the level of cytosine methylation at selected sites of the DNA based on the gene panel in biological material of at least one diseased individual with an unknown **disease** or medical condition, for generating a second knowledge base; (d) providing a third knowledge base comprising expert rules for comparing the first and second knowledge base; and (e) selecting a type of **disease** or medical condition for the at least one diseased individual with an unknown **disease** or medical condition based on the first to third knowledge bases. Steps (b)-(d) are repeated 100 times before performing step (e). The identical or different biological material, or their combination is used in step

(e). Treating a **disease** or medical condition comprises: (a) providing at least one diagnosis; and (b) installing a specific treatment for the at least one diagnosed **disease** or medical condition. The specific treatment is **disease** specific and/or personalized. The analysis of the expression level of the at least one gene in the biological sample comprises determining the relative amount of mRNA or protein derived from said at least one gene. The analysis comprises one- or **two**-dimensional gel **electrophoresis**, differential display, analysis of selected sets of turnout **markers**, subtractive hybridization, mass spectrometry, comparative expressed sequence tag sequencing, representational difference analysis, cDNA or oligonucleotide arrays, serial analysis of gene expression, enzymatic, fluorescent, radioactive, dye and/or antibody labeling, while measuring intensities of expression. The expression levels of at least 100 genes are analyzed in parallel. The analysis of the level of cytosine methylation comprises chemical treatment with bisulfite, hydrogen sulfite or disulfite, polymerase chain reaction (PCR), hybridization analyses, sequencing, mass spectrometry and fluorescent, enzymatic, radioactive, dye and/or antibody labeling. The analysis is at least partially performed by means of a suited automate, e.g. robot. The selection is based on a combination of the analysis of both mRNA level and protein expression or on the result of at least **two** individual rows of analyses. The selection is performed in such a way as to give a first or second knowledge base comprising only one set or different subsets of selected genes. The selection is at least partially performed automatically by means of a suited automate, such as a computer device. At least **2** or 100 genes are selected in parallel. Preferred Gene Panel: The gene panel is in the form of a knowledge base on a computer disc, RAM, ROM or a printed table or listing. The gene panel comprises additional information about methylation relevant regions, such as the complete genes and/or promoters, introns, first exons and/or enhancers of the genes to be analyzed. All or part of the genes of the second knowledge base is added to the gene panel. Additional information about methylation relevant regions of the selected genes is added to the gene panel.

ACTIVITY - Cytostatic; Neuroprotective; Nootropic; Antiinflammatory; Analgesic; Hypotensive; Neuroleptic; Cardiant. No biological data is given.

MECHANISM OF ACTION - None given.

USE - The method is useful for diagnosing or treating unwanted side effects of medicaments, cancers, dysfunctions, damages or diseases of the central nervous system, aggressive symptoms or behavioral disorders, clinical, psychological and social consequences of brain injuries, psychotic disorders and disorders of the personality, dementia and/or associate syndromes, cardiovascular diseases, respiratory system, gastrointestinal, skin, muscular, sexual, connective tissue or bone malfunctions or damages, injury, inflammation, infection, immunity and/or reconvalescence diseases, malfunctions or damages as consequences of modifications in the developmental process, endocrine or metabolic diseases, headache or their combinations, leukemia, head and neck cancer, Hodgkin's **disease**, gastric cancer, prostate cancer, renal cancer, bladder cancer, breast cancer, Burkitt's lymphoma, Wilms tumor, Prader-Willi/Angelman syndrome, ICF syndrome, dermatofibroma, **hypertension**, pediatric neurobiological diseases, autism, ulcerative colitis, fragile X syndrome or Huntington's **disease**. (84 pages)

L7 ANSWER 5 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2003-03819 BIOTECHDS
TITLE: New isolated polynucleotide sequences expressed in chondrocytes from a fetus, normal individual or patients with **osteoarthritis** useful as **markers** for constructing chondrocyte-specific microarrays for diagnosing **osteoarthritis**;

recombinant protein production, expressed sequence tag and DNA array for pharmacogenomics

AUTHOR: LIEW C; MARSHALL W E; ZHANG H
PATENT ASSIGNEE: CHONDROGENE INC
PATENT INFO: WO 2002070737 12 Sep 2002
APPLICATION INFO: WO 2002-CA247 28 Feb 2002
PRIORITY INFO: US 2001-305340 13 Jul 2001; US 2001-271955 28 Feb 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-698758 [75]
AN 2003-03819 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Isolated polynucleotide sequences selected from a fully defined list of names of all the expressed sequence tags (EST) identified in the four cDNA libraries that represents each of the 5807 known genes (F1), given in the specification, are new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a vector comprising the novel isolated polynucleotide; (2) a host cell comprising the vector in (1); (3) a composition comprising one or more chondrocyte enriched or chondrocyte-specific polynucleotide sequences isolated from one or more of fetus, normal, mildly osteoarthritic, moderately osteoarthritic, markedly osteoarthritic or severely osteoarthritic cartilage samples; (4) compositions comprising one or more polynucleotide sequences selected from any of the 4 fully defined lists of 13398 (F2), 16970 (F3), 12651 (F4) or 14222 (F5) names of EST sequences, which are respectively identified from the library constructed from fetal cartilage tissue, normal cartilage tissue obtained less than 14 hours post-mortem, cartilage of patients with mild **osteoporosis**, and cartilage of patients with severe **osteoporosis**, and whose sequences are disclosed in a CD-ROM given in the specification; (5) compositions comprising one or more polynucleotide sequences, where at least one of the polynucleotide sequences is differentially expressed in: (a) cartilage from a patient diagnosed with mild, severe, marked or moderate **osteoporosis** relative to cartilage derived from a normal individual isolated from cartilage tissue obtained less than 14 hours post-mortem, or to cartilage isolated from a fetus; (b) cartilage from normal individual relative to cartilage isolated from fetus; or (c) cartilage isolated from any **two** or more of the following sources: fetus; patients with mild, moderate, marked, or severe **osteoarthritis**; or cartilage isolated from a normal individual isolated from cartilage tissue obtained less that 14 hours post-mortem; (6) compositions comprising one or more polynucleotide sequences of: (a) upregulated genes detected in the mild or severe **osteoarthritis** cDNA library based on the microarray analysis respectively comprising 36 and 38 EST names, and/or (F1), fully identified in the specification; (b) (F1) which corresponds to the genes disclosed in lists of 855 and 957 genes, fully defined in the specification, which are respectively expressed between fetal/normal and mild/severe **osteoarthritis** cDNA libraries; (c) (F1), which correspond to 5807 genes, fully defined in the specification; or (d) one or more of the sequences of the list of 330 EST sequence names fully defined in the specification; (7) arrays comprising: (a) nucleic acid members, where at least one member has a unique position on the array and is differentially expressed in: (i) a cartilage from a patient diagnosed with mild, severe, moderate or marked **osteoarthritis**; or a fetus, as compared to cartilage form a normal individual; or (ii) cartilage isolated from any **two** or more of the following sources: fetus; patients with mild, moderate, marked, or severe **osteoarthritis**; or cartilage isolated from a normal individual; and (b) a solid substrate, where the at least one nucleic acid member is stably associated; (8) diagnosing mild, moderate, marked or severe **osteoarthritis** in a patient; (9) identifying an agent that increases or decreases the expression of a polynucleotide sequence that is differentially expressed in fetal developmental stage or in mild,

moderate, marked or severe osteoarthritic chondrocyte **disease**; (10) preparing a chondrocyte cDNA library; (11) making an array comprising nucleic acid members selected from a list of EST sequences in a CD ROM, not defined in the specification, on a solid support having a surface of pre-selected unique regions; and (12) a kit comprising any of the arrays of (11) and a packaging means.

BIOTECHNOLOGY - Preferred Array: The array comprises the nucleic acid differentially in the above-mentioned cartilages as compared to that of the normal individual, which is preferably living. The cartilage from this normal individual is isolated from the cartilage tissue less than 14 hours post-mortem. The nucleic acid member is at least 50 nucleotides. The array comprises 10-20000 positions. The array further includes negative and positive control sequences and RNA quality control sequences from cDNA sequences encoded by housekeeping genes, plant gene sequences, bacterial sequences, polymerase chain reaction (PCR) products or vector sequences. Preferred Method: Diagnosing mild, moderate, marked or severe **osteoarthritis** in a patient comprises hybridizing a nucleic acid sample corresponding to an array cited above, where the cartilage isolated from the normal individual is isolated from cartilage tissue less than 14 hours post-mortem. The hybridization of the nucleic acid sample to one or more differentially expressed nucleic acid members is indicative of **osteoarthritis**. The method further comprises isolating RNA from the patient; or a cartilage, blood or synovial fluid samples, and preparing a nucleic acid sample corresponding to the RNA. Identifying an agent that increase or decreases the expression of a polynucleotide sequence that is differentially expressed in fetal developmental stage or in mild, moderate, marked or severe osteoarthritic chondrocyte **disease**, comprises: (a) incubating a chondrocyte derived from a normal individual with a candidate agent, where the chondrocyte is isolated from a cartilage sample obtained less that 14 hours post-mortem; (b) isolating RNA from the chondrocyte; and (c) hybridizing a probe to the RNA. The probe corresponds to a polynucleotide sequence differentially expressed in a chondrocyte derived from fetal, normal, or mild, moderate, marked or severe osteoarthritic. The differential hybridization of the probe of the probe to the RNA from normal individual relative to one or more of the samples obtained from the sources cited above is indicative of the level of expression of RNA corresponding to a differentially expressed chondrocyte-specific polynucleotide sequence. As a result of the incubation step in the presence of the candidate agent, a change in the level of expression of the polynucleotide sequence is inactive of an agent that increases or decreases the expression of chondrocyte-specific polynucleotide sequence. Preparing a chondrocyte cDNA library comprises: (a) isolating chondrocytes from one or more fetuses, or from a cartilage sample derived from one or more normal individuals, or patients diagnosed with mild, moderate, sever or marked **osteoarthritis**; (b) isolating mRNA from the chondrocytes; (c) synthesizing cDNA from the mRNA; and (d) ligating the cDNA into a vector. Making an array comprising nucleic acid members selected from a list of EST sequences in a CD ROM, not defined in the specification, on a solid support having a surface of pre-selected unique regions, comprises: (a) spotting each nucleic acid member individually onto a unique pre-selected region; and (b) stably attaching each nucleic acid member to the solid support.

USE - The novel polynucleotide sequences are useful as **markers** for constructing human chondrocyte-specific microarrays to generate gene expression for detecting and diagnosing **osteoarthritis**. The agents identified in the method above are useful for treating alleviating **osteoarthritis** symptoms such as pain, swelling, weakness and loss of functional ability in the inflicted joints. The arrays are also useful for differential analysis of tissues and cartilages and in pharmacogenomics.

EXAMPLE - cDNA libraries were constructed into lambdaTripleEx2 vector through a polymerase chain reaction (PCR)-based method using switching mechanism at 5' end of RNA transcript (SMART) cDNA Library

Construction Kit. Phage plagues were randomly picked and positive inserts were identified by PCR. Agarose gel **electrophoresis** was used to assess the presence and purity of inserts. PCR product was then subjected to automated DNA sequencing with a 5' vector-specific forward primer and sequenced by ABI PRISM 3700 DNA Analyzer. A total for 13398 expressed sequence tags (EST) from human fetal cartilage, 17151 from normal cartilage, 12651 from mild osteoarthritic and 14222 from severe osteoarthritic cDNA libraries were obtained.(777 pages)

L7 ANSWER 6 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2003-05386 BIOTECHDS
TITLE: New PRO842 polypeptides having structural homology to interleukin-8, useful for treating or diagnosing a mammal with an inflammatory **disease** or immune related **disease**, e.g. rheumatoid arthritis, **osteoarthritis** or allergic **disease**; vector-mediated gene transfer and expression in host cell for recombinant protein production, drug screening and gene therapy
AUTHOR: FRENCH D; GRIMALDI J C; HILIAN K J; PISABARRO M T; SCHMIDT K N; SMITH V; TUMAS D; VANDLEN R L; WATANABE C K; WILLIAMS P M; WOOD W I
PATENT ASSIGNEE: GENENTECH INC
PATENT INFO: WO 2002070706 12 Sep 2002
APPLICATION INFO: WO 2001-US48060 7 Dec 2001
PRIORITY INFO: US 2001-941992 28 Aug 2001; WO 2001-6520 28 Feb 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-750461 [81]
AN 2003-05386 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - An isolated polypeptide having at least 80 % amino acid sequence identity to a 119 residue amino acid sequence, given in the specification, optionally lacking its signal peptide, a sequence of an extracellular domain of it, with its signal peptide, a sequence of an extracellular domain of it, lacking its signal peptide, or a sequence encoded by the full-length coding sequence of the cDNA deposited under ATCC accession number 203004, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated nucleic acid having at least 80 % sequence identity to: (a) a nucleotide sequence encoding any of the sequences of the polypeptide cited above; (b) an 870 base pair sequence, given in the specification; (c) a sequence comprising the full-length coding sequence of (b); or (d) the full-length coding sequence of the cDNA deposited under ATCC accession number 203004; (**2**) a vector comprising the nucleic acid of (1); (3) a host cell comprising the vector of (**2**); (4) a process for producing a PRO842 polypeptide; (5) a chimeric molecule comprising the polypeptide fused to a heterologous amino acid sequence; (6) an antibody that specifically binds to the novel polypeptide; (7) a composition of matter comprising the polypeptide, its agonist or antagonist, or the antibody of (6), and a carrier; (8) an article of manufacture comprising a container, a label on the container, and the composition of matter of (7), contained within the container, where the label on the container indicates that the composition of matter can be used for treating an immune related **disease**; (9) treating an immune related disorder in a mammal by administering to the mammal the polypeptide, its agonist or antagonist, or the antibody of (6); (10) determining the presence of a PRO842 polypeptide in a sample suspected of containing the polypeptide; (11) diagnosing an immune related **disease** in a mammal; (12) identifying a compound that inhibits the activity of a PRO842 polypeptide; (13) identifying a compound that inhibits the expression of a gene encoding a PRO842 polypeptide; (14) identifying a compound that mimics the activity of a PRO842 polypeptide; and (15) detecting the presence of tumor in a mammal.

BIOTECHNOLOGY - Preparation: Producing a PRO842 polypeptide comprises culturing the host cell under conditions suitable for expression of the polypeptide and recovering the polypeptide from the cell culture (claimed). Preferred Vector: The vector is operably linked to control sequences recognized by a host cell transformed with the vector. Preferred Host Cell: The host cell is a Chinese hamster ovary (CHO) cell, an Escherichia coli cell, a yeast cell, or a Baculovirus-infected insect cell. Preferred Chimeric Molecule: The heterologous amino acid sequence is an epitope tag sequence or an Fc region of an immunoglobulin. Preferred Antibody: The antibody is a monoclonal antibody, a humanized antibody or a single-chain antibody. Preferred Composition: The polypeptide, its agonist or antagonist, or the antibody of (6) is capable of increasing the proliferation of T-lymphocytes in a mammal, inhibiting the proliferation of T-lymphocytes in a mammal, increasing infiltration of inflammatory cells into a tissue of a mammal, or decreasing the infiltration of inflammatory cells into a tissue of a mammal. Preferred Method: Determining the presence of a PRO842 polypeptide in a sample suspected of containing the polypeptide comprises exposing the sample to an anti-PRO842 antibody and determining binding of the antibody to a component of the sample. Diagnosing an immune related **disease** in a mammal, comprises detecting the level of expression of a gene encoding PRO842 polypeptide in a test sample of tissue cells obtained from the mammal, and in a control sample of known normal tissue cells of the same cell type, where a higher or lower level of expression of the gene in the test sample as compared to the control sample is indicative of the presence of an immune related **disease** in the mammal from which the test tissue cells were obtained. Alternatively, the method comprises contacting an anti-PRO842 antibody with a test sample of tissue cells obtained from the mammal and detecting the formation of a complex between the antibody and the polypeptide in the test sample, where formation of the complex is indicative of the presence of an immune related **disease** in the mammal from which the test tissue cells were obtained. Identifying a compound that inhibits the activity of a PRO842 polypeptide comprises contacting cells that normally respond to the polypeptide with the polypeptide and a candidate compound, and determining the lack responsiveness by the cell to the polypeptide. Identifying a compound that inhibits the expression of a gene encoding a PRO842 polypeptide comprises contacting cells that normally express the polypeptide with a candidate compound, and determining the lack of expression of the gene. The candidate compound is an antisense nucleic acid. Identifying a compound that mimics the activity of a PRO842 polypeptide comprises contacting cells that normally respond to the polypeptide with a candidate compound, and determining the responsiveness by the cell to the candidate compound. Detecting the presence of tumor in a mammal, comprises comparing the level of expression of any PRO842 polypeptide given in the specification, in a test sample of cells taken from the mammal and a control sample of normal cells of the same cell type, where a higher level of expression of the PRO842 polypeptide in the test sample as compared to the control sample is indicative of the presence of tumor in the mammal. The tumor is lung tumor, colon tumor, or breast tumor.

ACTIVITY - Antiinflammatory; Dermatological; Hepatotropic; Antiallergic; Antiasthmatic; Immunosuppressive; Antithyroid; Antidiabetic; Antianemic; Hemostatic; Antipsoriatic; Antirheumatic; Antiarthritic; Nephrotropic. No biological data is given.

MECHANISM OF ACTION - Interleukin Agonist 8; Interleukin Antagonist 8; Gene Therapy.

USE - The composition is useful for treating or diagnosing a mammal with an inflammatory **disease** or immune related **disease** , e.g. systemic lupus erythematosus, rheumatoid arthritis, **osteoarthritis**, juvenile chronic arthritis, spondyloarthropathy, systemic sclerosis, idiopathic inflammatory myopathy, Sjogren's syndrome, systemic vasculitis, sarcoidosis, autoimmune hemolytic anemia, autoimmune thrombocytopenia, thyroiditis, **diabetes** mellitus,

immune-mediated renal **disease**, demyelinating **disease** of the central or peripheral nervous system, idiopathic demyelinating polyneuropathy, Guillain-Barre syndrome, chronic inflammatory demyelinating polyneuropathy, hepatobiliary **disease**, infectious or autoimmune chronic active hepatitis, primary biliary cirrhosis, granulomatous hepatitis, sclerosing cholangitis, inflammatory bowel **disease**, gluten-sensitive enteropathy, Whipple's **disease**, an autoimmune or immune-mediated skin **disease**, a bullous skin **disease**, erythema multiforme, contact dermatitis, psoriasis, an allergic **disease**, asthma, allergic rhinitis, atopic dermatitis, food hypersensitivity, urticaria, an immunologic **disease** of the ovaries, an immunologic **disease** of the lung, eosinophilic pneumonia, idiopathic pulmonary fibrosis, hypersensitivity pneumonitis, a transplantation associated **disease**, graft rejection or graft-versus-host-**disease** (claimed). The polypeptides are useful as molecular weight **markers** for protein **electrophoresis** purposes. The nucleic acids can be used as hybridization probes, in chromosome and gene mapping and in the generation of antisense RNA and DNA. PRO842 can also be used for preparing PRO842 polypeptides by recombination techniques, or in generating transgenic animals or knock out animals used in developing and screening therapeutically useful reagents.

ADMINISTRATION - Normal dosage is 10 ng/kg-100 mg/kg/day, preferably 1-10 micro-g/kg/day. Administration may be injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial or intralesional routes, topical administration or by sustained release systems.

EXAMPLE - The DNA encoding a PRO842 polypeptide was initially amplified using selected polymerase chain reaction (PCR) primers. The PCR-amplified, poly-His tagged sequences were ligated into an expression vector that was used to transform an Escherichia coli host. Transformants were first grown in Luria Broth (LB) containing 50 mg/ml carbenicillin at 30 degrees C with shaking until an O.D.600 of 3-5 was reached. Cultures were then diluted 50-100 fold into CRAP media (3.57 g (NH4)SO4, 0.71 g sodium citrate.2H2O, 1.07 g KCl,. 5.36 Difco yeast extract, 5.36 Sheffield hycase SF in 500 mL water, 110 mM MPOS (undefined) pH 7.3, 0.55 % (w/v) glucose and 7 mM MgSO4) and grown for approximately 20-30 hours at 30 degrees C with shaking. Samples were removed to verify expression by sodium dodecyl sulfate-polyacrylamide gel **electrophoresis** (SDS-PAGE) analysis, and the bulk culture was centrifuged to pellet the cells. Cell pellet were frozen until purification and refolding. The proteins were refolded by diluting the sample slowly into freshly prepared refolding buffer. Fractions containing the desired folded PRO842 polypeptide were pooled and the acetonitrile removed using a gentle stream of nitrogen directed at the solution. (118 pages)

L7 ANSWER 7 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2003-01630 BIOTECHDS
TITLE: Herpes simplex virus-based amplicon vector has large capacity cloning vector, herpes virus origin of replication, cleavage/packaging signal and genomic DNA fragment, and infects/delivers genomic DNA to **target** cell; virus vector expression in host cell, bacterium artificial chromosome and site-specific recombination use in **disease** therapy and gene therapy
AUTHOR: CHIOCCA E A; SAEKI Y; WADE-MARTINS R
PATENT ASSIGNEE: GEN HOSPITAL CORP
PATENT INFO: WO 2002053576 11 Jul 2002
APPLICATION INFO: WO 2002-US97 4 Jan 2002
PRIORITY INFO: US 2001-330511 23 Oct 2001; US 2001-259697 5 Jan 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-599605 [64]
AN 2003-01630 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - A herpes simplex virus (HSV)-based amplicon vector (I) comprising a large capacity cloning vector, a herpes virus origin of replication, a herpes virus cleavage/packaging signal and a genomic deoxyribonucleic acid (DNA) fragment, and is capable of infecting and delivering the genomic DNA to a **target** cell, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) constructing (M1) a HSV-based amplicon vector carrying a genomic DNA fragment, involves subcloning a genomic DNA fragment into a cloning vehicle comprising a large capacity cloning vector, a herpes virus origin of replication, and a herpes virus cleavage/packaging signal, such that the HSV-based amplicon vector is capable of infecting and delivering the genomic DNA to a **target** cell; (2) a rapid system for producing viral vectors containing transgenes of interest, comprising **two** site-specific recombination events between a plasmid comprising a viral genome and a prokaryotic backbone, and a transfer plasmid comprising a transgene of interest, where site-specific recombination event occurs in bacteria and the other site-specific recombination event occurs in mammalian cells; and (3) an infectious, expression-ready genomic DNA library (II) for use in functional genomics comprising several vectors, each comprising a large capacity cloning vector, a herpes virus origin of replication, a herpes virus cleavage/packaging signal, and a genomic DNA fragment, where the vectors are capable of propagating within bacterial cells and packaging into infectious particles.

BIOTECHNOLOGY - Preferred Vector: The large capacity cloning vector is a cosmid, yeast artificial chromosomal (YAC), mammalian artificial chromosome (MAC), human artificial chromosome, viral-based vector, preferably bacterial artificial chromosome (BAC) or P1 phage-based vector (PAC). Preferred Library: The herpes virus origin of replication is HSV-1 origin of replication, and herpes virus cleavage/packaging signal is an HSV-1 cleavage/packaging signal. The genomic DNA fragment is derived from a human, mouse or rat chromosome.

ACTIVITY - Neuroprotective; Nootropic; Antiparkinsonian; Antidiabetic; Antilipemic.

MECHANISM OF ACTION - Gene therapy. No suitable data given.

USE - (I) is useful for converting a large capacity cloning vehicle containing genomic DNA into a HSV-based amplicon comprising HSV-1 alone, or HSV-1 and Epstein-Barr virus (EBV), such that the HSV-based amplicon infects and delivers the genomic DNA to a **target** cell, by recombining (I) with the large capacity cloning vehicle. The recombining of (I) is accomplished using site-specific recombination, especially loxP/Cre recombinase-mediated recombination, homologous recombination, or ligation of DNA. The genomic DNA is non-human genomic DNA or human genomic DNA which contains a gene that encodes a therapeutic protein or hypoxanthine phosphoribosyltransferase (HPRT) or low density lipoprotein receptor (LDLR). The human genomic DNA contains human or mammalian centromeric DNA for creating human or mammalian artificial chromosomes, and regulatory or controlling DNA sequences. The genomic DNA is 50-100 or 100-150 kb in size. (II) is useful for isolating a genomic DNA clone encoding a gene product with a preselected function, by obtaining (II), packaging the vectors into infectious particles, infecting host cells (mammalian cells) with the infectious particles, identifying an infected host cell that exhibits a phenotype indicative of preselected function, and isolating the genomic DNA fragment of the library vector from the infected host cell. The method further comprises determining nucleotide sequence of isolated genomic DNA fragment (all claimed). (I) facilitates the delivery of genomic DNA to a **target** cell, and is useful for infecting model cells (human or mouse) which permit the identification of the preselected function. (II) is useful in functional genomics. Constructing HSV-based amplicon vector carrying a genomic DNA fragment contained in the Bacterial artificial chromosome (BAC) or P1 artificial chromosome (PAC) that encodes a therapeutic protein is useful for treating various diseases. The proteins includes neurotransmitter

biosynthetic enzymes e.g. tyrosine hydroxylase for treating Parkinson's **disease**; neurotrophic factors including neurotrophins, e.g. nerve growth factor for treating Alzheimer's **disease**; nerve growth factor receptor and trk receptor, hypoxanthine-guanine phosphoribosyl transferase for treating Lesch Nyhan **disease**; and beta-hexosaminidase alpha chain for treating Tay Sachs **disease**, insulin **diabetes**, hypercholesterolemia.

ADMINISTRATION - Administered at a dose of 1 pg/ml-20 mug/ml, preferably 0.1-10 mug/ml, by intravenous, parenteral, intraperitoneal, oral or subcutaneous route or by using catheters.

ADVANTAGE - (I) provides a excellent platform for the delivery of BAC and PAC inserts, as it has a high transgene capacity of 150 kb, high titer amplicon stocks can be produced by helper virus-free packaging systems, and resulting virion particles have a broad cell tropism across a wide range of species. Converting a large capacity cloning vehicle containing genomic DNA into HSV based amplicon using (I), is more efficient and easy than conventional methods.

EXAMPLE - Conversion of bacterial artificial chromosome (BAC) or P1 artificial chromosome (phage-based vector) (PAC) library clone into a Herpes simplex virus-1/Epstein-Barr virus (HSV-1/EBV) hybrid amplicon was performed. Electro-competent DH10B Escherichia coli cells were prepared carrying each parental BAC or PAC clone. Each E.coli line was electroporated with 10 ng of pEHHG (which contained a single loxP site, and R6Kgamma bacterial replication origin) and 10 ng of pCTP-T (a helper plasmid that supplied pir protein) and incubated for 1 hr at 30degreesC in SOC medium containing 20 mug/ml heat-inactivated chlortetracycline (cTc). Each bacterial culture was then diluted 1:10 into Luria-Bertani (LB) medium containing 20 mug/ml cTc and the appropriate antibiotics, and incubated. Pulsed-field gel **electrophoresis** (PFGE) was used to identify recombinant clones which contained only a single copy of the retrofitting pEHHG cassette. Each of the six constructs was purified and packaged into HSV-1 virions. Amplicon stocks were titered by assaying green fluorescent protein (GFP) expression on a 293 confluent cell layer. Typically, yields of 106 transducing units/ml were obtained in concentrated stocks. Undigested deoxyribonucleic acid (DNA) prepared from the packaged amplicons were analyzed by PFGE. Six amplicons were used to infect the SV40-immortalized human male lung fibroblast cell line MRC-5V2 at an multiplicity of infection (MOI) of 1, based on the titers calculated on 293 cells. It was found that the MRV-5V2 cells were infected, with upwards of 90% of cells transduced, as assayed by GFP expression. **Two** assays were performed to assess the ability of the infectious HSV-1 virion particles to deliver intact DNA to MRC-5V2 fibroblast cells by infection. First, the recircularization of amplicon DNA following infection was assayed by Gardella gel **electrophoresis**. Circular, supercoiled DNA was seen in transduced cells 48 hours after infection with the HSV-1 amplicons up to 153 kb in size. No supercoiled DNA was seen following infection with pHSV-176. Second, the genomic DNA was prepared from transduced MRC-5V2 cells 48 post-infection. A plasmid rescue assay was performed to assess the efficiency with which the amplicons delivered the BAC or PAC insert intact. The results indicated that all the amplicons of 100-153 kb were delivered intact with an efficiency of 25-100%. A library BAC clone containing the complete 45 kb locus of the human low density lipoprotein receptor (LDLR) contained within a 135 kb genomic DNA insert was retrofitted with **two** HSV-1 amplicon vectors. The vector pHG was used to convert the LDLR BAC into HSV-1 amplicon, and then the vector pEHHG was used to convert the LDLR BAC into a HSV-1/EBV hybrid amplicon. Both amplicons were used to infect MRC-5V2 cells at an MOI of approximately 1. The infections were 50-75% efficient, as judged by GFP reporter gene expression. Plasmid rescue on the infected MCR-5V2 cells revealed the pHSV-LDLR and pHSV/EBV-LDLR amplicons to be rescued intact with 75% and 57% efficiency, respectively. The pHSV-LDLR amplicon was used to infect Chinese Hamster ovary (CHO) ldlr-/-a7 cells expressing the Herpes virus entry protein C (HveC). 72 hours post-infection, vector

delivery was measured by GFP expression and delivery of a functional LDLR was assayed using uptake Dil-LDL, a fluorescently labeled form of LDL. The strong DiI fluorescence indicated expression from the genomic LDLR locus carried by pHSV-LDLR.(80 pages)

L7 ANSWER 8 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-18895 BIOTECHDS
TITLE: Identifying single nucleotide polymorphisms at defined positions in **target** nucleic acids, by utilizing oligonucleotide primers that contain a part of an interrupted restriction endonuclease recognition sequence; polymerase chain reaction and restriction enzyme for SNP detection and **disease** diagnosis
AUTHOR: VAN NESS J; GALAS D J; GARRISON L K
PATENT ASSIGNEE: KECK GRADUATE INST
PATENT INFO: WO 2002046447 13 Jun 2002
APPLICATION INFO: WO 2000-US30742 2 Oct 2000
PRIORITY INFO: US 2001-301394 27 Jun 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-527924 [56]
AN 2002-18895 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Identifying (I) a nucleotide at a defined position in a single stranded **target** nucleic acid (tNA), utilizes a pair of oligonucleotide primers (ODNPs) comprising a nucleotide sequence that is complementary to a nucleotide sequence of tNA or complementary to a nucleotide sequence of the complement of tNA and further comprising part of an interrupted restriction endonuclease recognition sequence.

DETAILED DESCRIPTION - Identifying (I) a nucleotide at a defined position in a single stranded **target** nucleic acid (tNA), utilizes a pair of oligonucleotide primers (ODNPs) comprising a nucleotide sequence that is complementary to a nucleotide sequence of tNA or complementary to a nucleotide sequence of the complement of tNA and further comprising part of an interrupted restriction endonuclease recognition sequence. (I) involves: (a) forming a mixture of a first oligonucleotide primer (ODNP), a second ODNP and a tNA, where the first ODNP comprises a nucleotide sequence that is complementary to a nucleotide sequence of a tNA at a location 3' to the defined position, the second ODNP comprises a nucleotide sequence that is complementary to a nucleotide sequence of the complement of tNA at a location 3' to the complementary nucleotide of the nucleotide at the defined position, and the first and second ODNPs further comprise a first constant recognition sequence (CRS) of a first strand and a second CRS of a second strand of an interrupted restriction endonuclease recognition sequence (IRERS), respectively, but not a complete IRERS, the complete IRERS being a double-stranded nucleic acid having the first and the second strands and comprising the first and the second constant recognition sequences (CRS) linked by a variable recognition sequences (VRS); (b) extending the first and second ODNPs to form a fragment having the complete IRERS where the nucleotide to be identified is within the VRS; (c) cleaving the fragment with a restriction endonuclease that recognizes the complete IRERS; and (d) characterizing a product to thereby determine the identity of the nucleotide at the defined position. INDEPENDENT CLAIMS are also included for the following: (1) an oligonucleotide primer (II), comprising: (a) a first CRS of a first strand of an IRERS, but not the first strand of a complete IRERS, and (b) at a location 5' to the 5' terminus of the first CRS, an oligonucleotide sequence complementary to a nucleotide sequence of a single stranded tNA at a location 3' to a defined position, where when the oligonucleotides sequence anneals to tNA, the distance between the nucleotide in the **target** corresponding to the 3' terminal nucleotide of the primer and the defined position is within the range 0 to n-1; (2) a oligonucleotide primer pair (III) for producing a portion of a single-stranded tNA containing a nucleotide to be identified

at the defined position, comprising first and second ODNP as above, and a fragment resulting from amplification of the first and second ODNPs comprising a complete IRERS, where the nucleotide to be identified is within the VRE; (3) a composition comprising (II) or (III), and a tNA; (4) a kit comprising (III); (5) a set of **two** ODNP pairs, comprising first and second ODNP pairs each comprising first and second ODNPs, where: (a) the first ODNP in the first ODNP pair comprises an oligonucleotide sequence complementary to a nucleotide sequence of a single stranded tNA at a location 3' to a defined position in tNA and a first CRS of a first strand of an IRERS, but not the first strand of a complete IRERS; (b) a second ODNP in the first ODNP pair comprises an oligonucleotide sequence complementary to a nucleotide sequence of tNA at a location 5' to a defined position and a second CRS of the first strand of the IRERS but not the first strand of the complete IRERS; (c) the first ODNP in the second ODNP pair comprises an oligonucleotide sequence complementary to a nucleotide sequence of the complement of tNA at a location 5' to the position in the complement corresponding to the defined position in tNA, and a first CRS of the second strand of the IRERS, but not the second strand of a complete IRERS; (d) the second ODNP in the second ODNP pair comprises an oligonucleotide sequence complementary to a nucleotide sequence of the complement of tNA at a location 3' to the position in the complement corresponding to the defined position in tNA, and a second CRS of the second strand of the IRERS, but not the second strand of a complete IRERS; and(e) a fragment resulting from an extension and ligation of the first and second ODNPs in each ODNP pair comprises the complete IRERS where the nucleotide to be identified is within the VRS; and (6) determining (IV) the identity of a nucleotide of interest by, providing a double stranded nucleic acid molecule comprising an IRERS, which comprises a first CRS and a second CRS linked by VRS having a nucleic acid of interest, cleaving the nucleic acid molecule with a restriction with a endonuclease that recognizes the IRERS and characterizing at least one of the products to determine the identity of the nucleotide of interest.

BIOTECHNOLOGY - Preferred Method: The single stranded tNA is one strand of a denatured double-stranded nucleic acid. The double-stranded nucleic acid is genomic nucleic acid, or cDNA. The single stranded tNA is derived from genome of a pathogenic virus, or the genome or episome of a pathogenic bacterium. tNA is synthetic nucleic acid. The nucleotide sequence of the first, and second ODNP corresponding to tNA is at least 12 nucleotides in length. The first and second ODNP is 15-85 nucleotides in length. The first ODNP further comprises one or more nucleotides complementary to tNA at the 3' terminus of the first CRS, and the second ODNP further comprises one or more nucleotides complementary to tNA at the 3' terminus of the second CRS. Extension is performed by polymerase chain reaction. The extended product is characterized by mass spectrometry, liquid chromatography, fluorescence polarization, electron ionization, gel **electrophoresis**, or capillary **electrophoresis**. All the steps are performed in a single vessel. The IRERS is recognizable by a restriction endonuclease such as BslI, MwoI, or XcmI. (IV) comprises forming a mixture of first ODNP, second ODNP, and single-stranded **target**, extending the first and second ODNPs, ligating the extended products and amplifying the ligation product with an oligonucleotide which has a universe nucleotide at the position corresponding to the defined position in tNA and the resulting double stranded nucleic acid molecule comprising an IRERS. Preferred Kit: The kit further comprises a restriction endonuclease that recognizes the IRERS, and instructions for use.

USE - (I) is useful for identifying a nucleotide at a defined position in a single-stranded tNA. The defined position may be polymorphic or associated with a **disease**, especially a human genetic **disease**, or drug resistance of a pathogenic microorganism (claimed). The defined position is associated with a **disease**, including a human genetic **disease** e.g. bladder carcinoma, sickle-cell anemia, thalassemias, Alzheimer's **disease**

, phenylketonuria, galactosemia, Wilson's **disease**, **diabetes** insipidus, familial hypercholesterolemia, or neurofibromatosis. (I) finds applications in genetic analysis for hereditary **disease**, tumor diagnosis, **disease** predisposition, forensics or paternity, crop cultivation and animal breeding, expression profiling of cell function and/or **disease marker** genes, and identification and/or characterization of infectious organisms that cause infectious **disease** in plants or animals and/or that are related to food safety. The method is useful for determining gene variations in T-cell receptor genes encoding variable, antigen-specific regions that are involved in the recognition of various foreign antigens.

EXAMPLE - A specific sequence from the human genome was amplified in which the primers contained the BslI restriction endonuclease recognition sequence. The resulting amplicon contained a cutting site that liberated a **two** double-strand oligonucleotide fragment, which was then subjected to a chromatography step and identified by mass to charge ratio. The PCR reactions (50 microlitres) were composed of genomic DNA (25 ng), 0.5 microMolar each forward and reverse primers, Tris (10 mM) pH 8.3, KCl (50 mM), MgCl2 (1.5 mM), 200 microMolar each dNTP, and DNA polymerase (1 unit). After the thermocycling was complete, an enzyme mixture was prepared containing BslI and 10x BslI buffer. The mixture was added to each well to make final concentrations of KCl (150 mM), Tris-HCl (10 mM), MgCl2 (**2** mM), dithiothreitol (DTT) (1 mM), pH 7.5. The reaction was carried out at 55 degrees Centigrade for more than 60 minutes. The chromatography system was an Varian Prostar Helix system. The cytochrome 2D6 gene containing a specific single nucleotide polymorphism was tested and successfully separated and identified. **Two** primer pairs were used for amplifying the region of cytochrome 2D6 gene containing the single nucleotide polymorphism (SNP). The external primers were designed to amplify only cytochrome 2D6 gene, and not its pseudogenes. The internal primers were designed to have a partial BslI recognition sequence and to amplify a small region of cytochrome 2D6 gene containing the SNP. External forward and reverse primers were 5'-GAGACCAGGGGGAGCATA-3', and 5'-GGCGATCACGTTGCTCA-3', and the internal forward and reverse primers were 5'-TGGGCCTGGATGCTAAGTCGCTGGCCCAG-3', and 5'-GGCCTCCTCGGTCCCCC-3'. The final amplification product was then digested by BslI and denatured. One of the digestion product had the sequence TGGGCCTGGATGCTAAGTCGCTGGCCCAGTG. The mass of this sequence, including a 3' OH and 5' PO4 was 9993.**2** amu (atomic mass units).(82 pages)

L7 ANSWER 9 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2003-02165 BIOTECHDS
TITLE: Novel schizophrenia-related voltage-gated ion channel polypeptide and polynucleotide useful for identifying modulators and for diagnosing, treating schizophrenia, bipolar disorder or central nervous system disorders; vector-mediated recombinant CanIon protein gene transfer and expression in mammal host cell for cardiovascular **disease** diagnosis, prognosis and gene therapy
AUTHOR: COHEN D; CHUMAKOV I; SIMON A; ABDERRAHIM H
PATENT ASSIGNEE: GENSET
PATENT INFO: WO 2002046404 13 Jun 2002
APPLICATION INFO: WO 2001-IB2798 4 Dec 2001
PRIORITY INFO: US 2000-251317 5 Dec 2000; US 2000-251317 5 Dec 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-619018 [66]
AN 2003-02165 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - An isolated, purified, or recombinant CanIon polypeptide (a schizophrenia-related voltage-gated ion channel polypeptide) (I) comprising a sequence (S1) of 1738 amino acids as defined in the

specification, or its biologically active fragment, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated purified or recombinant CanIon polynucleotide (II) comprising a nucleotide sequence (S2) of 82293 (genomic sequence comprising 5' regulatory region and exons 1-7), 237961 (genomic sequence comprising exons 8-27), 47841 (genomic sequence comprising exons 28-44 and the 3' regulatory region), 6799 (cDNA sequence), or 453 (amplicon sequence comprising biallelic **marker** A18) bp given in the specification or its complement; (**2**) an isolated, purified or recombinant polynucleotide (III) comprising a contiguous span of at least 50 nucleotides of the cDNA sequence of CanIon of 6799 bp given in the specification, where the polynucleotide encodes a biologically active CanIon polypeptide; (3) an isolated purified or recombinant polynucleotide (IV) which encodes (I) or its biologically active fragment; (4) an array of polynucleotides comprising at least one polynucleotide as above; (5) a recombinant vector (V) comprising a polynucleotide as above optionally, operably linked to a promoter; (6) a host cell comprising (V); (7) a non-human host animal or mammal comprising (V); (8) a mammalian host cell or non-human host mammal comprising a CanIon gene disrupted by homologous recombination with a knock out vector;(9) making (I); (10) binding an anti-CanIon antibody to (I), by contacting the antibody with the polypeptide under conditions in which the antibody can specifically bind to the polypeptide; (11) detecting (VI) the expression of a CanIon gene within a cell, by contacting the cell or an extract from the cell with, a polynucleotide that hybridizes under stringent conditions to (II), (III) or (IV), or a polypeptide that specifically binds to (I) and detecting the presence or absence of hybridization between the polynucleotide and an RNA species within the cell or extract, or the presence or absence of binding of the polypeptide to a protein within the cell or extract, where a detection of the presence of the hybridization or of the binding indicates that the CanIon gene is expressed within the cell; (12) identifying (VII) a candidate modulator of a CanIon polypeptide, by contacting the polypeptide with a test compound and determining whether the compound specifically binds to the polypeptide, where a detection that the compound specifically binds to the polypeptide indicates that the compound is candidate modulator of the CanIon polypeptide; and (13) preparing a pharmaceutical composition, where the modulator identified by (VII) is combined with a carrier.

WIDER DISCLOSURE - Also disclosed are: (1) variants, fragments, analogs and derivatives of (I), including mutated CanIon proteins; (**2**) variants and fragments of (II); (3) isolated or recombinant polypeptide comprising a sequence having at least 70-99% identity with (S1); (4) an isolated antibody capable of specifically binding (I), mutated CanIon protein or its fragment; (5) T-cell antigen receptor is specifically bind (I); (6) antibodies that are anti-idiotypic to the above antibodies; (7) composition comprising (I) fused or conjugated to antibody domain other than variable domains; (8) purified and/or isolated nucleotide sequence comprising a polymorphic base of a CanIon-related biallelic **marker**; (9) diagnostic kits comprising one or more polynucleotides; and (10) compounds that interact with, bind to or activate or inhibit the expression or activity (I) or (II).

BIOTECHNOLOGY - Preparation: (I) is prepared by providing a population of cells comprising a polynucleotide encoding (I), operably linked to a promoter, culturing the population of cells under conditions conductive to the production of the polypeptide within the cells and purifying the polypeptide from the population of cells (claimed). Preferred Polynucleotide: (II), (III) or (IV) is attached to a solid support. Preferred Array: The array is addressable and further comprises a label. Preferred Method: In (VI), the polynucleotide is an oligonucleotide primer, and hybridization is detected by detecting the presence of an amplification product comprising the sequence of the primer. The polypeptide that binds to (I) is an anti-CanIon antibody. (VII) further comprises testing the voltage gated ion channel activity of

the CanIon polypeptide in the presence of the candidate modulator, where a difference in the activity of the CanIon polypeptide in the presence of the candidate modulator in comparison to the activity in the absence of the candidate modulator indicates that the candidate modulator is a modulator of the CanIon polypeptide. The polypeptide is present in a cell or cell membrane.

ACTIVITY - Neuroleptic; Hypotensive; Anticonvulsant; Analgesic; Antiarrhythmic; Antianginal; Cardiant; Antimanic; Antidepressant. No supporting biological data is given.

MECHANISM OF ACTION - Gene therapy; Modulator of (I) or (II). No supporting biological data is given.

USE - (I) is useful for identifying a candidate modulator of a CanIon polypeptide (claimed). (I) and (II) are useful in the treatment of schizophrenia, bipolar disorder, or other central nervous system (CNS) conditions, as well as other conditions such as heart conditions and **hypertension**. Compounds that block CanIon channels are useful to treat a number of diseases or conditions, preferably schizophrenia or bipolar disorder, and also including pain disorders, epilepsy and various cardiovascular disorders such as heart arrhythmias, angina and **hypertension**. (I) is useful to generate antibodies capable of specifically binding to an expressed CanIon protein. The biallelic **markers** are useful for genetic analysis of complex traits, for identifying if the CanIon gene is associated with a detectable trait, estimating the frequency of a haplotype for a set of biallelic **markers** in a population and estimating the frequency of an allele in a population. The biallelic **markers** are also useful to identify patterns of biallelic **markers** associated with detectable traits resulting from polygenic interactions and in transmission/disequilibrium test. The CanIon nucleic acid sequence and biallelic **markers** are useful to develop diagnostic tests capable of identifying individuals who express a detectable trait as the result of a specific genotype or individuals whose genotype places them at risk of developing a detectable trait at a subsequent time. Such as diagnosis can be useful in the staging, monitoring, prognosis and/or prophylactic or curative therapy of schizophrenia, bipolar disorder and other CNS disorders, pain disorders and cardiovascular conditions.

ADMINISTRATION - Administered by oral, rectal, transmucosal, intestinal or parenteral route. Dosage not specified.

EXAMPLE - DNA from 100 individuals was extracted and tested for the detection of the biallelic **markers**. Thirty ml of peripheral venous blood were taken from each donor in the presence of ethylenediaminetetraacetic acid (EDTA). The amplification of specific genomic sequences of the DNA samples was carried out on the pool of DNA. Each pair of first primers was designed using the sequence information of the CanIon gene (schizophrenia-related voltage-gated ion channel gene), the OSP software. The synthesis of these primers was performed following the phosphoramidite method. DNA amplification was performed. The quantities of the amplification products obtained were determined on 96-well ml plates, using a fluorometer and Picogreen as intercalant agent. The amplified products were sequenced. The products of the sequencing reactions were run on sequencing gels and the sequences were determined using gel image analysis. The sequence data were further evaluated to detect the presence of biallelic **markers** within the amplified fragments. The polymorphism search was based on the presence of superimposed peaks in the **electrophoresis** pattern resulting from different bases occurring at the same position as described previously. In the 17 fragments of amplification, 18 biallelic **markers** were detected.(272 pages)

L7 ANSWER 10 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-19609 BIOTECHDS
TITLE: Novel osteoblast polypeptide, designated OBP1 and polynucleotide encoding it, useful for treating bone disorders e.g. **osteoporosis**, **osteoarthritis**

, osteomalacia, Maroteaux-Lamy syndrome, and as diagnostic **markers**;
recombinant protein production and agonist and antagonist use in **disease** therapy and gene therapy

AUTHOR: LI Y P; CHEN W
PATENT ASSIGNEE: FORSYTH DENTAL INFIRMARY FOR CHILDREN
PATENT INFO: WO 2002044377 6 Jun 2002
APPLICATION INFO: WO 2000-US44756 28 Nov 2000
PRIORITY INFO: US 2000-724304 28 Nov 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-527711 [56]
AN 2002-19609 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - An isolated osteoblast polypeptide (I), designated OBP1, comprising all or a portion of a sequence (S1) of 78 amino acids fully defined in the specification, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated nucleic acid molecule (II) encoding (I), and comprising or consisting essentially of a nucleotide sequence (S2) of 1083 base pairs (bp) defined in the specification, nucleotides 319-555 of (S2), or their complement; (**2**) a vector (III) comprising (II) operatively linked to a regulatory sequence; (3) a recombinant host cell (IV) comprising (III); (4) producing (I); (5) an isolated polypeptide (V) encoded by (II); and (6) an antibody (VI) or its antigen-binding fragment which selectively bins to (I) or (V).

WIDER DISCLOSURE - Also disclosed are: (1) an isolated polypeptide comprising an amino acid sequence greater than 80% identical to (S1); (**2**) nucleic acid constructs containing (II); (3) fragments and variants of (I) encoded by (II); (4) chimeric or fusion proteins of (I); (5) antibodies to fragments of (I); (6) kits for detecting the presence of (I) or (II) in the biological sample; (7) modulating expression or activity of genes or proteins; (8) assays for screening candidate or test compounds that bind to or modulate the activity of (I); and (9) novel agents identified by the screening methods.

BIOTECHNOLOGY - Preparation: (I) is produced by culturing (IV) under conditions suitable for expressing the nucleic acid molecule (claimed). Preferred Nucleic Acid: An isolated nucleic acid molecule which hybridizes to (II) under high stringency conditions is also preferred. (II) is a DNA.

ACTIVITY - Osteopathic; Cytostatic; Metabolic; Immunosuppressive; Antirheumatic; Antiarthritic; Anti-tumor; Anoretic; Gastrointestinal; Cardiovascular. No supporting data is given.

MECHANISM OF ACTION - Gene therapy.

USE - (II) is useful for assaying the presence of nucleic acid molecule in a sample. (VI) is useful for assaying the presence of (I) in a sample (claimed). (I) is useful as molecular weight **markers** on sodium dodecyl sulfate-polyacrylamide gel **electrophoresis** (SDS-PAGE) gels or on molecular sieve gel filtration columns, to raise antibodies or to elicit an immune response, and as labeled reagent in assays to quantitatively determine levels of the protein or molecule to which it binds in biological fluids. (I) is also useful as **markers** for cell or tissues in which the corresponding protein is expressed, to isolate binding partner e.g. receptor or ligand, and to screen for peptide or small molecule antagonists or agonists of the binding interaction. (II) is useful as probes or primers in hybridization assays, to hybridize and discover related DNA sequences or to subtract out known sequences from the sample, as molecular weight **markers** on Southern gels, and as chromosome **markers**. (II) is also useful to derive primers for genetic fingerprinting, to raise anti-protein antibodies, as antigen to raise anti-DNA antibodies or elicit immune response, to identify and express recombinant proteins for analysis, characterization or therapeutic use, or as **markers** for tissues in which the corresponding protein is expressed, either

constitutively, during tissue differentiation, or in diseased states. (II) is also useful for producing and identifying nucleic acid probes useful for identifying OBP1 DNA, in gene therapy, and to cause OBP1 expression in cells in which such expression does not ordinarily occur, e.g. in cells which were not osteoblasts. (II) is useful as reagents in chromosomal mapping, to tissue typing and in forensic identification of a biological sample. (II) is useful for both prophylactic and therapeutic treatment of a subject at risk of or susceptible to a disorder or having a disorder associated with aberrant expression or activity of the proteins or nucleic acid molecules. Diseases treatable include **osteoporosis**, osteosclerosis, pyknodysotosis, osteomyelosclerosis, hyperphosphatasia, progressive diaphyseal dysplasia, melorheostosis, osteopoikilosis, hyperostosis frontalis interna, sclerotosis, McCune-Albright syndrome and spondyloepiphyseal dysplasia, disorders characterized by a decrease in bone density, including **osteoarthritis**, Maroteaux-Lamy syndrome and **osteoporosis** , and disorders of bone formation, including osteogenisis imperfecta, sutural craneosynostosis, osteomalacia and cleidocranial dysplasia and bone cancers such as osteosarcomas; bone disorders associated with cancer, anorexia, nervosa and autoimmune disorders; and diseases or systemic conditions affecting bone in which abnormal osteoblastic cell function/activity e.g. acromegaly, hypercalcemia, primary or secondary hyperparathyroidism, rheumatoid arthritis, multiple myeloma and bone metastases of various tumors. (I), (II) and (VI) are useful for identifying osteoblasts using well established techniques. (IV) is useful for producing non-human transgenic animals which are useful for studying the function and/or activity of the nucleotide sequence and polypeptide encoded by the sequence and for identifying and/or evaluating modulators of their activity. (VI) is useful to isolate polypeptide, to facilitate purification of polypeptide from cells, to detect the polypeptide, to evaluate the abundance and pattern of expression of the polypeptide, and diagnostically to monitor protein levels in tissue as part of a clinical testing procedures, for e.g. determining the efficacy of a given treatment regimen.

EXAMPLE - Identification of sequences of osteoblast designated, OBP1 using a subtractive differential screening method was as follows: 5 microg of E9.5 embryo head mRNA (i.e., the source of the pre-osteoblast) was reverse-transcribed using random primers. A reaction was carried out at 42 degrees C for 1 hour with 32P-dCTP incorporation. After precipitation, reaction products were resuspended in 0.1M NaOH and incubated for 20 minutes at 65 degrees C to hydrolyze RNA templates. Probes were neutralized with 0.1 acetic acid and size fractionated on Sephadex G-50. Biotinylated RNA as driver was prepared from E9.5 embryo body without the head. cDNA probes were hybridized with a 10-fold excess of biotinylated mRNA. The precipitated cDNA-RNA mixtures were resuspended in 10-20 microl of water and heated. An equal volume of 2X hybridization buffer (12X sodium saline citrate (SSC) containing 1% sodium dodecyl sulfate) was added, and the mixture was incubated. Following addition of an equal volume of 4-(**2**-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES) buffer, streptavidin (20 microg) was added and the mixture was incubated on ice. Biotinylated RNA and RNA-cDNA duplexes, complexed with avidin, were removed by repeated phenol-chloroform extractions. Aqueous-phase cDNA probes were precipitated with ethanol and subjected to a second round of subtraction. A total of 5x10 power 5 clones from osteoblast cDNA library were screened. Duplicate filters were made from 145 mm plates containing 1x10 power 4 recombinant bacteriophage each by plaque lifts, and these were hybridized in parallel using equal amounts of the subtracted probes or the control probes that were derived from the animal trunk. The filters were hybridized, washed and exposed to films. Head-specific clones were identified by overlaying films from corresponding filters. Clones selected in the primary screening were re-screened once at low density to verify differential expression and for plaque purification. OBP1 cDNA clones were sequenced using an ABI377 sequencer. Nucleotide sequences

were compared by the BLAST algorithm with known sequences. A nucleotide sequence identified was 1083 (bp) base pairs, that encoded a protein of 78 amino acids defined in the specification. Northern hybridization was used to confirm the results from subtractive-differential screening, and to determine the tissue and cellular distribution, size and amount of OBP1. (41 pages)

L7 ANSWER 11 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-19600 BIOTECHDS
TITLE: Novel Zyctor19 polypeptides and polynucleotides useful for stimulating immune responses in animals for producing antibodies, and for treating autoimmune diseases, leukemia and asthma;
recombinant protein, fusion protein and antibody for use in diagnosis and therapy and as a recombinant vaccine
AUTHOR: PRESNELL S R; XU W; NOVAK J E; WHITMORE T E; GRANT F J
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PATENT INFO: WO 2002044209 6 Jun 2002
APPLICATION INFO: WO 2000-US44808 28 Nov 2000
PRIORITY INFO: US 2001-267211 7 Feb 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-527700 [56]
AN 2002-19600 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - An isolated Zyctor19 polypeptide (I) comprising amino acids (a.as) 21(R)-223(P), 21(R)-226(N), 21(R)-249(W), 250(K)-491(R), 21(R)-491(R) or 1(M)-491(R) of a sequence (S1) comprising 491 a.as, a.as 250(K)-520(R), 21(R)-520(R) or 1(M)-520(R) of a sequence (S2) comprising 520 a.as, or a.as 21(R)-163(W), 21(R)-211(S) or 1(M)-211(S) of a sequence (S3) comprising 211 a.as, fully defined in the specification, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a polynucleotide (IIa) encoding (I); (2) a DNA construct (IIb) encoding a fusion protein, comprising a first segment encoding (I) and at least one other DNA segment encoding an additional polypeptide, where the first and the other DNA segments are connected in-frame, and they together encode the fusion protein; (3) an expression vector (III) comprising a transcription promoter, (IIa) or (IIb), and a transcription terminator, where the promoter is operably linked to (IIa) or (IIb), and (IIa) or (IIb) is operably linked to the transcription terminator; (4) a cultured cell (IV) comprising (III), where the cell expresses a polypeptide encoded by the DNA segment or DNA construct; (5) producing a fusion protein, by culturing (IV) and isolating the polypeptide produced by the cell; (6) producing (I); (7) an isolated polypeptide comprising an amino acid segment, comprising amino acids 21(R)-226(N) of S1, 21(R)-211(S) of S3, a sequence (S4) comprising 203 amino acids fully defined in the specification, or a sequence having at least 90% identity to the above said sequences, where the polypeptide is substantially free of transmembrane and extracellular domains ordinarily associated with hematopoietic receptors;(8) producing an antibody to a polypeptide; (9) an antibody (Ab) produced by the above said method, which specifically binds to S1, S2 or S3, or which binds specifically to (I); (10) detecting a cytokine receptor ligand within a test sample; (11) detecting a genetic abnormality in a patient; and (12) detecting a cancer in a patient.

BIOTECHNOLOGY - Preparation: (I) Is obtained by culturing (IV) and isolating the polypeptide produced by the cell (claimed). Preferred Sequence: (II) Encodes a polypeptide that further comprises a transmembrane domain consisting of residues 227(W)-249(W) of S1 and intracellular domain consisting of residues 250(K)-491(R) of S1, 250(K)-520(R) of S2. The polypeptide has the activity as measured by cell proliferation, activation of transcription of a reporter gene, or where the polypeptide encoded by (II) further binds to an antibody which is raised to (I). The binding of the antibody to (I) is measured by a biological or biochemical assay including radioimmunoassay,

radioimmuno-precipitation, Western blot or enzyme immunosorbant assay. (III) further comprises a secretory signal sequence operably linked to the DNA segment. The cell expresses a polypeptide encoded by the DNA segment. Preferred Cell: (IV) Expresses a soluble receptor polypeptide encoded by the DNA segment.

ACTIVITY - Immunosuppressive; cytostatic; antirheumatic; antiarthritic; neuroprotective; antidiabetic; antiinflammatory; nephrotropic; dermatological; anti-HIV; hemostatic.

MECHANISM OF ACTION - Modulator of cytokine receptor protein; vaccine (claimed). No supporting data given.

USE - (IV) Is useful for detecting the presence of a modulator of the activity of a cytokine receptor protein. Ab Is useful for detecting cancer in a patient (claimed). (I) Or Ab is useful for suppressing immune system for reducing rejection of tissue or organ transplants and grafts and for treating T-cell specific leukemias or lymphomas and autoimmune diseases including rheumatoid arthritis, multiple sclerosis, **diabetes** mellitus, inflammatory bowel **disease** and Crohn's **disease**. Ab Is useful for treating immunologic renal diseases, glomerulonephritis, mesangioproliferative **disease**, chronic lymphocytic leukemia, secondary glomerulonephritis or vasculitis associated with lupus, polyarteritis, scleroderma, HIV-related diseases, amyloidosis and hemolytic uremic syndrome. (I) and Ab is useful for renal or urological neoplasms and multiple myelomas, asthma, bronchitis, emphysema and other chronic airway diseases. Ab Is also useful as vaccines. (I) Is useful for raising Ab and for identifying modulators of (I). (I) Is also useful as an aid to teach preparation of antibodies, identifying proteins by Western blotting, protein purification, determining the weight of expressed Zcytor19 polypeptides as a ratio of total protein expressed, identifying peptide cleavage sites, coupling amino and carboxyl terminal tags, amino acid sequence analysis, for teaching analytical skills, and for monitoring biological activities of both the native and tagged protein in vitro and in vivo. Ab Is also useful as a teaching aid to prepare affinity chromatography columns to purify Zcytor19, cloning and sequencing the polynucleotide encoding Ab, and as a practicum for teaching humanized antibody design. Ab Is useful for screening biological samples in vitro for the presence of Zcytor19, and for isolating DNA sequences that encode human Zcytor19 genes from cDNA libraries. Ab Is useful for tagging cells that express zcytor19, for isolating zcytor19 by affinity purification, for diagnostic assays for determining circulating levels of zcytor19 polypeptides, for detecting or quantitating soluble zcytor19 as **marker** of underlying pathology or **disease**, for detecting or quantitating in a histologic biopsy and for stimulating cytotoxicity and for screening expression libraries.

ADMINISTRATION - 20-100 mg, preferably 50-70 mg of Ab is administered through intravenous, intraarterial, intraperitoneal, intramuscular, subcutaneous, intrapleural, intrathecal or direct intralesional route.

EXAMPLE - Human Multiple Tissue Northern Blots (Human 12-lane MTN Blot I and II, and human Immune System MTN Blot II) were probed to determine the tissue distribution of human zcytor19 expression. A polymerase chain reaction (PCR) derived probe that hybridizes to a sequence comprising 1476 or 1563 nucleotides fully defined in the specification was amplified using standard PCR amplification methods. The PCR product was visualized by agarose gel **electrophoresis** and the PCR product was gel purified. The probe was radioactivity labeled and purified. EXPRESSHYB (RTM) solution was used for pre-hybridization and as hybridizing solution for the Northern blots. The blots were washed, and after exposure to X-ray film, a transcript corresponding to a length of 1476, 1563 or 674 nucleotides fully defined in the specification or of an mRNA encoding a sequence of 491, 520 or 211 amino acids fully defined in the specification was expected to be seen in tissues that specifically express zcytor19, but not other tissues. Northern analysis was also performed using Human Cancer Cell Line MTN (RTM). A strong signal in a

cancer line suggested that zcytor19 expression is expressed in activated cells and/or indicated a cancerous **disease** state. Moreover, Northern blots or PCR analysis of activated lymphocyte cells showed the expression of zcytor19 in activated immune cells. Based on electronic Northern information, zcytor19 was shown to be expressed specifically in pre-B cell acute lymphoblastic leukemia cells. (200 pages)

L7 ANSWER 12 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-17942 BIOTECHDS
TITLE: Diagnosing **diabetes** or susceptibility to **diabetes** in individual by typing insulin receptor gene region or insulin receptor protein in sample obtained from individual;
insulin receptor protein region typing, and transgenic animal model use in **disease** therapy and diagnosis
AUTHOR: HOSFORD D; PURVIS I J
PATENT ASSIGNEE: GLAXO GROUP LTD
PATENT INFO: WO 2002033121 25 Apr 2002
APPLICATION INFO: WO 2000-GB4660 19 Oct 2000
PRIORITY INFO: GB 2000-25678 19 Oct 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-500014 [53]
AN 2002-17942 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Diagnosing (M1) **diabetes** or susceptibility to **diabetes** in an individual involves typing in a sample from the individual the insulin receptor gene region or the insulin receptor protein of the individual, and determining thereby whether the individual has **diabetes** or is susceptible to **diabetes**.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) treating (M2) a patient who has been diagnosed as having **diabetes** by (M1), involves administering to the patient: (a) an anti-**diabetes** agent (A1); or (b) an agent (A2) that prevents the development of **diabetes**; (2) use of (A1) or (A2), in the manufacture of a medicament for use in treating a patient who has been diagnosed as having **diabetes** by (M1); (3) treating or preventing (M3) **diabetes** by administering a compound (A3) of formula (I), (II) or (III), or its tautameric form, salt or solvate, to a patient who has an insulin resistance polymorphism, or polymorphism causing migraine, or a polymorphism which is in linkage disequilibrium with above mentioned polymorphism; (4) use of (A3) in the manufacture of medicament for treating or preventing **diabetes** in an individual who has a polymorphism as defined above; (5) a kit for diagnosing **diabetes** or susceptibility to **diabetes**, comprising an agent or a polynucleotide that is able to bind a polynucleotide containing the polymorphism, but which does not bind a polynucleotide with the corresponding wild-type sequence; (6) a non-human which has **diabetes** or is susceptible to **diabetes**, and which is transgenic for a polymorphism as defined above; and (7) identifying a polymorphism which can be typed to determine whether an individual has **diabetes** or is susceptible to **diabetes** involves determining whether a candidate polymorphism in the insulin receptor gene region or insulin receptor protein is associated with **diabetes** or susceptibility to **diabetes**, or is in linkage disequilibrium with a polymorphism that is associated with **diabetes** or susceptibility to **diabetes**, and thereby determining whether the polymorphism can be typed to determine whether an individual has **diabetes** or is susceptible to **diabetes**. A = phenyl optionally substituted with halogen atoms, 1-6C alkyl, 1-3C alkoxy, 1-3C fluoroalkoxy, nitrile and/or -NR7R8, 5- or 6-membered heterocyclic group containing at least one heteroatom of oxygen, nitrogen or sulfur or a fused bicylic ring of formula (Ia); R7 and R8 = hydrogen or 1-3C alkyl; B

= 1-6C alkylene, -M-1-6C alkylene- or 1-6C alkylene-M-1-6C alkylene-, 5- or 6-membered heterocyclic group containing at least one nitrogen heteroatom and optionally at lest one further heteroatom such as oxygen, nitrogen or sulfur and optionally substituted by 1-3C alkyl or Het-1-6C alkylene; M = O, S or -NR2; R2 = hydrogen or 1-3C alkyl; Alk = 1-3C alkylene; R1 = hydrogen or 1-3C alkyl; Z = -(1-3C alkylene) phenyl, which phenyl is optionally substituted by one or more halogen atoms; or -NR3R4; R3 = hydrogen or 1-3C alkyl; R4 = -Y-(C=O)-T-R5, or -Y-(CH(OH))-T-R5; Y = 5- or 6-membered heterocyclic group containing at least one nitrogen heteroatom and optionally at lest one further heteroatom such as oxygen, nitrogen or sulfur and optionally substituted by 1-3C alkyl; T = 1-3C alkyleneoxy, -O- or -N(R6)-; R6 = hydrogen or 1-3C alkyl; R5 = phenyl optionally substituted with halogen atoms, 1-6C alkyl, 1-3C alkoxy, 1-3C fluoroalkoxy, nitrile and/or -NR7R8; C = Hetcy, which bicyclic ring is attached to group B via a ring atom of ring C; D = Hetcy optionally substituted by (=O), which bicyclic ring is attached to T via a ring atom of ring D, or -1-6C alkylene MR11; M = O, S, or -NR12; R12 and R11 = hydrogen or 1-3C alkyl; R21 = hydrogen or 1-3C alkyl; R22 = hydrogen, or 1-8C alkyl optionally substituted by one or more halogens; R23 = group of formula (Ia); R' = hydrogen or 1-3C alkyl; R24 = 1-6C alkyl; R25 = hydrogen, halogen, or 1-3C alkyl optionally substituted by one or more halogens; R26 = hydrogen or 1-3C alkyl; X = O or S; n = 1, **2** or 3; R31 = H, 1-8C alkyl, amino(1-8C)alkyl, (1-8C)alkyl-amino-(1-8C)alkyl, heteroarylamino(1-8C)alkyl, (heteroaryl)(1-8C)alkyl-amino-(1-6C)alkyl, (4-8C cycloalkyl)-(1-8C)alkyl, 1-8C alkyl-heteroaryl-(1-8C)alkyl or 9-10 membered partially aromatic ring (optionally substituted); anddashed line = none or one double bond between the **two** carbon atoms.

WIDER DISCLOSURE - A pharmaceutical pack comprising (A1) or (A2) and instructions for administering the agents to humans diagnosed by (M1), is also disclosed.

BIOTECHNOLOGY - Preferred Method: In (M1), the polymorphism which is typed is an insulin resistance polymorphism, or a polymorphism which is in linkage disequilibrium with such a polymorphism. The above mentioned polymorphism (a) causes reduced binding of insulin to the insulin receptor, or (b) causes migraine, or is a polymorphism which is in linkage disequibilirium with such a polymorphism. The polymorphism which is preferably typed in (M1) is INSBa, INSCa, exon8.po11, exon11.po11, exon17.po12, exon6.po11, exon7.po11, exon 7.po12, exon8.po12, exon9.po13, exon14.po11 and INSR-c.4479C.T, or is in linkage disequilibrium with it. (M1) involves contacting polynucleotide or protein of the individual with a specific binding agent for the polymorphism and determining whether the agent binds to the polymorphism in the polynucleotide or protein, binding of the agent to the polymorphism indicating that the individual has **diabetes** or is susceptible to **diabetes**. The agent is a polynucleotide that is able to bind a polynucleotide containing the polymorphism, but which does not bind a polynucleotide with the corresponding wild-type sequence. Preferably, the polymorphism is detected by measuring the change caused by the polymorphism in the mobility of a polynucleotide or protein of the individual during gel **electrophoresis**. (M1) is most preferably carried out in vivo.

ACTIVITY - Antidiabetic.

MECHANISM OF ACTION - Reduces blood glucose levels; stimulates insulin release or reduces clearance of insulin. No supporting data is given.

USE - For diagnosing **diabetes** or susceptibility to **diabetes** in an individual. (M2) is useful for treating **diabetes** and (M3) is useful for treating and preventing **diabetes** (all claimed). The effectiveness of particular agents may be affected or dependent on whether the individual has particular polymorphisms in the insulin receptor gene region or insulin receptor. Thus (M1) allows determination of whether an individual will respond to a particular agent by determining whether the individual has a polymorphism which affects the effectiveness of that agent. Similarly, the method allows identification of a patient who is at increased risk of suffering

side effects due to such an agent by identifying whether an agent has such a polymorphism. The method may be used in the development of new drug therapies which selectively **target** one or more allelic variants of insulin receptor gene.

ADMINISTRATION - The active agents are administered by oral, parenteral, intravenous, intramuscular or subcutaneous route. Dosages range from 0.1-100 (preferably, 1-40) mg/kg body weight.

EXAMPLE - An association between polymorphisms in the insulin receptor gene and migraine was carried out as follows. Individuals with specific types of migraine: migraine without aura, migraine with aura or familial hemiplegic migraine were identified. Samples were obtained from the study group and genomic DNA extracted using a standard kit. The genotypes of the migraineurs with aura and control individuals for individual single nucleotide polymorphisms (SNPs) within the insulin receptor gene were then determined from the DNA samples obtained using the Taqman allelic discrimination assay. For each polymorphic site the allelic discrimination assay used **two** allele specific primers labeled with a different fluorescent dye at their 5' ends but with a common quenching agent at their 3' ends. Both primers had a 3' phosphate group so that Taq polymerase could not add nucleotides to them. The allele specific primers comprised the sequence encompassing the polymorphic site and differed only in the sequence at this site. The allele specific primes were only capable of hybridizing without mismatches to the appropriate allele. The allele specific primers were used in typing PCRs in conjunction with a third primer, which hybridized to the template 5' of the **two** specific primers. If the allele corresponding to one of the specific primers were present the specific primer would hybridize perfectly to the template. The Taq polymerase would then remove the nucleotides from the specific probe releasing both the fluorescent dye and the quenching agent. This resulted in an increase in the fluorescence from the dye no longer in close proximity to the quenching agent. If the allele specific primer hybridized to the other allele the mismatch at the polymorphic site would inhibit the 5' to 3' endonuclease activity of Taq and hence prevent release of the fluorescent dye. The ABI7700 sequence detection system was used to measure the increase in fluorescence from each specific dye during thermal cycling PCR directly in PCR reaction tubes. The information from the reactions was then analyzed. If an individual is homozygous for a particular allele only fluorescence corresponding to the dye from that specific primer would be released, if the individual is heterozygous both dyes would fluoresce. The P values for the co-inheritance of the associated SNPs with migraine e.g., INSB, INSC, X8pol1, X11pol1, X17pol2 were 0.002, 0.007, 0.018, 0.05, 0.008, respectively. (61 pages)

L7 ANSWER 13 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-12528 BIOTECHDS
TITLE: Method, oligonucleotides and arrays for parallel measurement of genetic variations, based on the incorporation of unique restriction endonuclease restriction sites flanking and encompassing genetic variation loci;
DNA array, restriction endonuclease restriction site, constant recognition sequence useful for mutation associated **disease** identification
AUTHOR: VAN NESS J; GALAS D J; GARRISON L K
PATENT ASSIGNEE: KECK GRADUATE INST
PATENT INFO: WO 2002029006 11 Apr 2002
APPLICATION INFO: WO 2000-US42432 2 Oct 2000
PRIORITY INFO: US 2001-301394 27 Jun 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-340099 [37]
AN 2002-12528 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Method, oligonucleotides and arrays for parallel measurement of

genetic variations, are new. The method is based on the incorporation of unique restriction endonuclease restriction sites flanking and encompassing genetic variation loci.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) a method (I) for identifying one or more nucleotide(s) at a defined position in a single-stranded **target** nucleic acid, comprising: (a) providing a first oligonucleotide primer (ODNP) immobilized to a substrate (the first ODNP comprises a nucleotide sequence complementary to a nucleotide sequence of the **target** nucleic acid at a location 3' to the defined position, and further comprises a first constant recognition sequence (CRS) of a first strand of an interrupted restriction endonuclease recognition sequence (IRERS), but not a complete lRERS, the complete IRERS being a double-stranded oligonucleotide having the first strand and a second strand and comprising the first and a second CRS linked by a variable recognition sequence (VRS)); (b) exposing the immobilized first ODNP to the **target** nucleic acid and a second ODNP (the second ODNP comprises a nucleotide sequence complementary to a nucleotide sequence of the complement of the **target** nucleic acid at a location 3' to the defined position of the **target** nucleic acid, and further comprises the second CRS of the second strand of the lRERS); (c) extending the first and second ODNPs so as to form a fragment having the complete lRERS (the nucleotide to be identified is within the VRS of the complete lRERS); (d) cleaving the fragment with a restriction endonuclease that recognizes the complete lRERS; and (e) characterizing a product of step (d) to determine the identity of the nucleotide to be identified; (**2**) an immobilized oligonucleotide primer (ODNP) (II), comprising: (a) an oligonucleotide sequence complementary to a nucleotide sequence of a single-stranded **target** nucleic acid at a location 3' to a defined position, the oligonucleotide sequence having 3' and 5' termini; and (b) at a location 3' to the oligonucleotide sequence of (a), a first constant recognition sequence (CRS) of a first strand of an interrupted restriction endonuclease recognition sequence (IRERS), but not a complete IRERS, the complete IRERS being a double-stranded oligonucleotide having the first strand and a second strand and comprising the first CRS and a second CRS linked by a variable recognition sequence (VRS); (3) a composition (III) comprising an immobilized oligonucleotide primer (ODNP) and a **target** nucleic acid, the ODNP is (II); (4) an array (IV) comprising: (a) a substrate having a number of distinct areas; and (b) a number of oligonucleotide primers (ODNPs) immobilized to the distinct areas, the ODNPs are (II); and (5) a kit (V) for genotyping comprising the array (IV).

BIOTECHNOLOGY - Preferred Methods: In (I) the defined position is polymorphic, and a mutation at the defined position is associated with a **disease**, e.g. bladder carcinoma, colorectal tumors, sickle-cell anemia, thalassemias, al-antitrypsin deficiency, Lesch-Nyhan syndrome, cystic fibrosis/mucoviscidosis, Duchenne/Becker muscular dystrophy, Alzheimer's **disease**, X-chromosome-dependent mental deficiency, and Huntington's chorea, phenylketonuria, galactosemia, Wilson's **disease**, hemochromatosis, severe combined immunodeficiency, alpha-1-antitrypsin deficiency, albinism, alkaptonuria, lysosomal storage diseases, Ehlers-Danlos syndrome, hemophilia, glucose-6-phosphate dehydrogenase disorder, agammaglobulimenia, **diabetes** insipidus, Wiskott-Aldrich syndrome, Fabry's **disease**, fragile X-syndrome, familial hypercholesterolemia, polycystic kidney **disease**, hereditary spherocytosis, Marfan's syndrome, von Willebrand's **disease**, neurofibromatosis, tuberous sclerosis, hereditary hemorrhagic telangiectasia, familial colonic polyposis, Ehlers-Danlos syndrome, myotonic dystrophy, osteogenesis imperfecta, acute intermittent porphyria, and von Hippel-Lindau **disease**. Alternatively, a mutation at the defined position may be associated with drug resistance of a pathogenic microorganism. The single-stranded **target** nucleic acid is one strand of a denatured double-stranded nucleic acid (genomic nucleic acid or cDNA) and is derived from the genome of a

pathogenic virus, or from the genome or episome of a pathogenic bacterium. The **target** nucleic acid may be a synthetic nucleic acid. The substrate comprises a material selected from silicon, glass, paper, ceramic, metal, metalloid, and plastics. The first ODNP: (i) is non-covalently immobilized to the substrate; (ii) has 31 and 5' termini and is covalently immobilized to the substrate at the 5' terminus; (iii) is prepared by photolithography; (iv) is synthesized on the substrate; and (v) is first synthesized and subsequently immobilized onto the substrate. The nucleotide sequence of the first ODNP that is complementary to the nucleotide sequence of the **target** nucleic acid is at least 12 nucleotides in length. The nucleotide sequence complementary to the nucleotide sequence of the complement of the **target** nucleic acid in the second ODNP is at least 12 nucleotides in length. Step (c) comprises performing a polymerase chain reaction (PCR). Step (d) produces a fragment with a 5' overhang, a blunt end, or 3' overhang. The restriction endonuclease is EcoN I. The nucleotide to be identified (or the complement of it) is within the 5' overhang. Step (e) further comprises filling a 3' recessed terminus corresponding to the 5' overhang with one or more nucleoside triphosphates, and/or washing the substrate before filling the 3' recessed terminus. The nucleoside triphosphate comprises a detectable label (a fluorophore and a radioisotope). The product of step (c) characterized in step (e) is or is not immobilized to the substrate. Step (e) is performed at least partially by the use of a technique selected from mass spectrometry, liquid chromatography, fluorescence polarization, electron ionization, gel **electrophoresis**, and capillary **electrophoresis**.
Preferred Primers: In the immobilized ODNP (II) the oligonucleotide sequence of (a) is at least 18 nucleotides in length. (II) Further comprises one or more nucleotides complementary to the **target** nucleic acid at a location 3' to the first CRS. The ODNP is non-covalently immobilized to the substrate, has 3' and 5' termini and is covalently immobilized to the substrate at the 5' terminus and is 15-80 nucleotides in length. The complete lRERS is recognizable by EcoN I. The defined position in the **target** nucleic acid is polymorphic, and the defined position in the **target** nucleic acid is associated with a **disease**. (II) Has regions A, B, C, D, E and F, and the ODNP being partially complementary to a **target** nucleic acid as shown in the diagram. A = an optional linking element that links the 5' end of the ODNP to a solid support (a polyether and/or a polyester); B = an optional nucleotide sequence (1 to 50 nucleotides); C = a nucleotide sequence (**2**-30 nucleotides) that is complementary to a nucleotide sequence of a single-stranded **target** nucleic acid at a location 3' to a defined position X of the **target** nucleic acid; D = a first constant recognition sequence (CRS) of a first strand of an interrupted restriction endonuclease recognition sequence (IRERS), but not a complete IRERS, the complete IRERS being a double-stranded oligonucleotide having the first strand and a second strand and comprising the first CRS and a second CRS linked by a variable recognition sequence (VRS) having a number n of variable nucleotides (**2**-6 nucleotides, especially sequence 5'-CCT-3'); E = an optional nucleotide sequence (1-8 nucleotides) complementary to the **target** nucleic acid; and F = an optional gap of nucleotides (1-8 nucleotides), where the number of nucleotides within regions E and F is within the range 0 to n-l.A, E And F may be present or absent, and A is cleavable. The number of nucleotides within regions B, C, D and E is between 15-80 nucleotides. The immobilization is non-covalent or covalent attachment to the solid support. In (III) X is a single nucleotide polymorphism (SNP), polymorphic and/or a mutation associated with a **disease** as described for (I). In (III) the ODNPs in anyone of the distinct areas are homogeneous, but different from the ODNPs in a second distinct area and/or the ODNPs in at least one of the distinct areas are heterogeneous. The ODNP is non-covalently or covalently immobilized to the substrate (preferably, it has 3' and 5' termini and is covalently immobilized to the substrate at the 5' terminus). The array ODNPs are prepared by

photolithography, synthesized on the substrate, are first synthesized and subsequently immobilized to the substrate. Each ODNP is 15-80 nucleotides in length (especially 12 nucleotides in length). At least one of the number of ODNPs further comprises one or more nucleotides complementary to the **target** nucleic acid at a location 31 to the first CRS. The defined position is polymorphic and a mutation at the defined position is associated with a **disease**. The complete lRERS is recognizable by EcoN I. 1000 to 1012 ODNP molecules are immobilized in at least one in the number of distinct areas. The substrate has 10-100 distinct areas, especially more than 1000 distinct areas. The substrate is made of silicon, glass, paper, ceramic, metal, metalloid, and plastic. The single-stranded **target** nucleic acid is one strand of a denatured double-stranded nucleic acid. The double-stranded nucleic acid is genomic DNA. The **target** nucleic acids are complementary to the ODNP(s) that comprise sequences are from one organism, or from **two** or more organisms of one species. The ODNP(s) in anyone of the distinct areas are the same as the ODNP(s) in a second distinct area. The surface of the array has raised portions to delineate the distinct areas. Preferred Kits: (V) Further comprises a restriction endonuclease that recognizes the complete IRERS, and/or a DNA polymerase.

USE - The method, primers and arrays may be used to identify mutations associated with a **disease**, e.g. bladder carcinoma, colorectal tumors, sickle-cell anemia, thalassemias, al-antitrypsin deficiency, Lesch-Nyhan syndrome, cystic fibrosis/mucoviscidosis, Duchenne/Becker muscular dystrophy, Alzheimer's **disease**, X-chromosome-dependent mental deficiency, and Huntington's chorea, phenylketonuria, galactosemia, Wilson's **disease**, hemochromatosis, severe combined immunodeficiency, alpha-1-antitrypsin deficiency, albinism, alkaptonuria, lysosomal storage diseases, Ehlers-Danlos syndrome, hemophilia, glucose-6-phosphate dehydrogenase disorder, agammaglobulimenia, **diabetes** insipidus, Wiskott-Aldrich syndrome, Fabry's **disease**, fragile X-syndrome, familial hypercholesterolemia, polycystic kidney **disease**, hereditary spherocytosis, Marfan's syndrome, von Willebrand's **disease**, neurofibromatosis, tuberous sclerosis, hereditary hemorrhagic telangiectasia, familial colonic polyposis, Ehlers-Danlos syndrome, myotonic dystrophy, osteogenesis imperfecta, acute intermittent porphyria, and von Hippel-Lindau **disease**. Alternatively, a mutation at the defined position may be associated with drug resistance of a pathogenic microorganism (claimed).

ADVANTAGE - The method offers a convenient, rapid and sensitive method for detecting mutations and parallel measurement of genetic variations. The methods exploit the high degree of specificity provided by restriction endonucleases and employ readily available detection techniques.

EXAMPLE - No suitable examples given.(135 pages)

L7 ANSWER 14 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-13128 BIOTECHDS
TITLE: Novel antisense oligonucleotide which inhibits expression of phosphorylase kinase beta, useful for treating metabolic disorder e.g. **diabetes**, prevent or delay infection, inflammation or tumor formation;
sense and antisense oligonucleotide and enzyme expression inhibition useful in **disease** gene therapy
AUTHOR: MONIA B P; WYATT J R
PATENT ASSIGNEE: ISIS PHARM INC
PATENT INFO: WO 2002022637 21 Mar 2002
APPLICATION INFO: WO 2000-US28586 14 Sep 2000
PRIORITY INFO: US 2000-662250 14 Sep 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-351873 [38]
AN 2002-13128 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - An antisense compound (I) of 8-30 nucleobases in length **targeted** to a nucleic acid molecule encoding phosphorylase kinase beta (PKB), and specifically hybridizes with and inhibits the expression of PKB, is new.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a pharmaceutical composition (PC) comprising (I).

BIOTECHNOLOGY - Preferred Antisense compound: (I) is an antisense chimeric oligonucleotide comprising at least one modified internucleoside linkage e.g. phosphorothioate linkage. (I) further comprises at least one modified sugar moiety e.g. **2**'-o-methoxyethyl sugar moiety, or one modified nucleobase e.g. 5-methylcytosine. Preferred Pharmaceutical Composition: PC further comprises a colloidal dispersion system.

ACTIVITY - Antidiabetic; antiinflammatory; cytostatic.

MECHANISM OF ACTION - Inhibitor of expression of PKB (claimed); antisense gene therapy. The human lung carcinoma cell line A549 were routinely cultured in Dulbecco's modified eagle medium (DMEM) basal media and passaged by trypsination and dilution until they reached 90% confluence. When the cells reached 80% confluence, they were treated with (I). After 4-7 hours of treatment the medium was replaced with fresh medium. Cells were harvested 16-24 hours after oligonucleotide treatment. The concentration of (I) was different for different cell lines. For human cell, the positive control oligonucleotide was ISIS 13920 (TCCGTCATCGCTCCTCAGGG), a **2**'-o-methoxyethyl gapmer with a phosphorothioate backbone which was **targeted** to human H-ras. The positive control oligonucleotide resulted in 80% inhibition of c-H-ras.

USE - (I) is useful for inhibiting the expression of PKB in cells or tissues, by contacting the cells or tissues with (I). (I) is also useful for treating a human having a **disease** or condition such as metabolic disorder e.g. **diabetes** associated with PKB expression (claimed). (I) is utilized for diagnostic, therapeutics, prophylactics, and as research reagents and kits. (I) is also useful to distinguish between functions of various members of a biological pathway. (I) is utilized prophylactically e.g. to prevent or delay infection, inflammation or tumor formation. (I) is also useful in the preparation of pharmaceutical formulation.

ADMINISTRATION - (I) is administered through topical, pulmonary, oral or parenteral routes at a dosage of 0.01 microgram-100 g/kg body weight.

ADVANTAGE - (I) is highly specific and is often preferred over native form because of enhanced cellular uptake, enhanced affinity for nucleic acid **target** and increased stability in presence of nucleases.

EXAMPLE - Chimeric oligonucleotides having **2**'-O-alkyl phosphorothioate and **2**'-deoxy phosphorothioate oligo-nucleotide segments were synthesized using an Applied Biosystems automated DNA synthesizer Model 380B. Oligonucleotides were synthesized using the automated synthesizer and **2**'-deoxy-5'-dimethoxytrityl-3'-O-phosphoramidite for the DNA portion and 5'-dimethoxytrityl-**2**'-O-methyl-3'-O-phosphoramidite for 5' and 3' wings. The standard synthesis cycle was modified by increasing the wait step after the delivery of tetrazole and base to 600 s repeated four times for RNA and twice for **2**'-O-methyl. The fully protected oligonucleotide was cleaved from the support and the phosphate group was deprotected in 3:1 ammonia/ethanol at room temperature overnight then lyophilized to dryness. Treatment in methanolic ammonia for 24 hours at room temperature was then done to deprotect all bases and sample was again lyophilized to dryness. The pellet was resuspended in 1M TBAF (undefined) in THF (undefined) for 24 hours at room temperature to deprotect the **2**' positions. The reaction was then quenched with 1 M TEAA (undefined) and the sample was then reduced to 1/**2** volume before being desalted on a G25 size exclusion column. The oligo recovered was then analyzed spectrophotometrically for yield and for purity by capillary

electrophoresis and by mass spectrometry. (**2**'-O-(**2**-methoxyethyl)-(**2**'-deoxy)-(-**2**'-O-(methoxyethyl)) chimeric phosphorothioate oligonucleotides were obtained. (132 pages)

L7 ANSWER 15 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-13279 BIOTECHDS
TITLE: Model system for modeling nitric oxide (NO)-mediated modulation of apoptosis in cells, involves identifying genes whose expression is modulated after modulating intracellular NO concentration in the cells;
HeLa cell gene and expressed sequence tag identification, and expression profiling
AUTHOR: COTTER T; DALY P; HAYES I; MURPHY F; SEERY L
PATENT ASSIGNEE: EIRX THERAPEUTICS LTD
PATENT INFO: WO 2002020834 14 Mar 2002
APPLICATION INFO: WO 2000-GB3969 4 Sep 2000
PRIORITY INFO: US 2000-254384 8 Dec 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-393848 [42]
AN 2002-13279 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - A model system (I) for modeling nitric oxide (NO)-mediated modulation of apoptosis in a cell comprising providing a population of cells, modulating the intracellular NO concentration in the cells, and identifying genes whose expression is modulated as a result of changes in intracellular NO concentration, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) modulating (M1) the expression of gene products that regulate the transition of a cell between a non-apoptotic state and an apoptotic state, involves modulating the intracellular concentration of NO; (**2**) identifying (M2) one or more gene product(s) that modulate the transition of a cell between a non-apoptotic and an apoptotic state, comprises exposing the cell to NO or to an agent which induces the production of NO in the cell or inhibits the production of NO in the cell, determining the level(s) of expression of one or more gene product(s) in a cell to establish a reference expression level, monitoring the level(s) of expression of the one or more gene product(s) in the cell, and identifying one or more of the gene product(s) whose expression has been increased, decreased or modified as a result of the NO modulation; (3) a use (II) of NO to induce the expression of gene products that modulate the transition of a cell from a non-apoptotic state to an apoptotic state; (4) identifying (M3) an inhibitor of apoptosis, involves identifying a compound which interacts with a gene identified by the system; and (5) an inhibitor (III) of apoptosis identified by (M3).

BIOTECHNOLOGY - Preferred Method: (M2) further comprises determining the level(s) of expression of one or more gene product(s) in a cell e.g. a HeLa cell, a neuron, or a cell with neuronal characteristics (e.g., PC-12), to establish a reference expression level, and comparing the expression level(s) after NO modulation to the reference expression level(s). Preferably, exposure of the cell to NO leads to induction of apoptosis in the cell more rapidly than it occurs under identical conditions but in the absence of NO. The expression levels of the one or more gene products are determined by: (a) assessing polypeptide production; (b) by assessing polypeptide post-translational modification; or (c) by assaying gene transcription. Assessing polypeptide production or polypeptide post-translational modification is determined by 2D-polyacrylamide gel **electrophoresis** (2D-PAGE) of one or more polypeptide populations. The cell is exposed to a chemical inducer of apoptosis, preferably sodium nitroprusside. Optionally, the cell is cultured in the presence of an inhibitor of apoptosis (e.g., N-acetyl-cysteine (NAC) or nerve growth factor (NGF)), which inhibitor

acts to delay the onset of apoptosis in the cell. The onset of apoptosis is monitored by morphological analysis, externalization of membrane phospholipid phosphatidyl serine or caspase activation analysis. The involvement of NO in the induction of the onset of apoptosis is measured by further exposing the cells to one or more NO inhibitors such as NOX 100, or L-NAME or other L-arginine analogues, L-Citrulline analogues, aminoguanidine, S-substituted isothioureas or bis-isothioureas, N (3aminomethyl) benzyl) acetamidine, 7-nitroindazole, trimethylphenylfluoroimidazole, mercaptoethyl, or mercaptopropyl-guanidines. The NO inhibitors are selective against specific isoforms of nitric oxide synthase (NOS), and thereby provide a system of identifying cell-specific genes involved in NO-mediated apoptosis signaling. The NOS-selective NO inhibitor is 7-nitroindazole such that neuronal specific genes involved in NO-mediated apoptotic signaling may be identified. More preferably the NOS-selective NO inhibitor is mercaptoethylguanidine or a mercaptopropyl-guanidine such that inflammatory cell specific genes involved in NO-mediated apoptotic signaling may be identified. The expression levels of the several gene product(s) as described above are determined by hybridization of one or more mRNA populations to a set of polynucleotides arrayed on to a substrate. Identifying an inhibitor of apoptosis, preferably involves modulating the intracellular NO concentration in the cell, identifying a gene whose expression is modulated as a result of changes in intracellular NO concentration, exposing the cell to a candidate compound, and identifying candidate compounds which modulate the expression of a gene identified in the above step.

ACTIVITY - Antiatherosclerotic; antiinflammatory; nootropic; neuroprotective; antiparkinsonian; cerebroprotective; cardiant. No suitable data given.

MECHANISM OF ACTION - Apoptosis modulator; modulator of expression of genes that modulate transition between non-apoptotic and apoptotic state of cell.

USE - (M1) is useful for modulating expression of gene products that regulate the transition of cell between a non-apoptotic and apoptotic state. (M2) is useful for identifying one or more gene products that regulate the transition of cell between a non-apoptotic and apoptotic state. The methods enable the identification of gene **targets** for therapeutic intervention in **disease** conditions such as septic shock, cerebral ischemia, neurodegenerative diseases, inflammatory diseases, inflammatory pain, migraine, **diabetes**, or meningitis (all claimed). (I) is useful for identifying, characterizing, cloning and validating molecules including oligonucleotides and polypeptides associated with apoptosis. The model enables the identification of genes which are involved in or cause early stage of apoptosis and also provides a screen for substances which interact with such genes to inhibit apoptosis. The compounds and substances identified may be used in treating diseases involving apoptosis, e.g. atherosclerosis, inflammatory conditions, systemic inflammatory response syndrome, Alzheimer's and Parkinson's **disease**, adult respiratory distress syndrome, stroke, myocardial infarction, etc. The system allows the study of transcription of individual mRNAs in the early induction of the commitment phase of apoptosis, and in particular following NO.

ADVANTAGE - The system enables identification and isolation of genes involved in early stages of apoptosis whose expression is modulated by reactive nitrogen species in the cell. The method also allows development of therapeutics to modulate and/or control apoptosis. The method overcomes a flaw associated with the methods currently used to manipulate the apoptotic process. The model system is capable of blocking the root cause of apoptosis by modulating the action of nitric oxide. It is capable of identifying genes which are involved in the earliest stages of apoptosis, before morphological signs of apoptosis appear. The system provides a method by which cell specific genes modulated by NO and involved in apoptotic signaling may be identified.

EXAMPLE - HeLa cells (HeLa-based model cell system) were obtained

from the ATCC and maintained in DMEM medium with 10% fetal calf serum (FCS) at 37 degrees Centigrade in a 5% CO2 atmosphere. HeLa cells were trypsinized, and made up to a concentration of 5x104 cells/ml. One hundred microlitres of cells were plated/well of a 96 well plate and allowed to adhere overnight. The following day cells were incubated with sodium nitroprusside (made up in DMEM; final conc 0.9x10-6 M) in the presence or absence of 20 mM N-acetyl-L-cysteine (NAC). Cell viability was measured by 3-(4,5-dimethylthiazol-2-yl)-2 ,5-diphenyltetrazolium bromide (MTT) assay. At 18 hours post-treatment the supernatant was removed and cells were washed in 2X serum free DMEM medium prior to adding 100 microl fresh medium containing 0.5 mg/ml MTT and allowed to incubate for a further 4 hours before developing the insoluble formazan products with dimethylsulfoxide (DMSO). Viability was positively correlated with the optical density at 570 nm. Treatment of HeLa cells with sodium nitroprusside induces cell death, as determined by a decrease in the ability of cultures treated with sodium nitroprusside to convert the soluble MTT to the insoluble purple formazan. Addition of NAC simultaneously with sodium nitroprusside significantly decreased the cytotoxicity of the nitric oxide (NO) donor, as determined by an increase in conversion in the conversion rates at 570 nm. Also HO2 (a reactive oxygen species (ROS)) induced HeLa cell death, which was reversed by NAC treatment. Following treatment of cultured HeLa cells with sodium nitroprusside, at a dose that induced apoptosis (0.9x10-6 M), total cellular RNA was isolated and examined for gene expression changes using microarray. Significant transcription of mRNA was observed. In one experiment, as many as 1169 genes were detected using Incyte human LifeGrid' filters; these genes were increased or decreased by greater than 2-fold over a 6 hour time course as compared with the time zero control HeLa cells. As many as 943 genes were increased or decreased as early as 4 hours post-treatment, and prior to the commitment to die' point identified as described. As many as 469 genes were increased or decreased by 6 hours post-treatment. By 6 host post treatment 184 genes were up regulated and 285 down-regulated. Many of these regulated genes were expressed sequence tags (ESTs) (e.g., 124 at 6 hours post-treatment) with little or no known biological function annotation. Sodium nitroprusside-induced HeLa cell apoptosis was inhibited by the addition of NAC (20 mM). Gene expression change most closely and reciprocally correlated with HeLa cell survival (with NAC), or death (without NAC), was likely to be casually involved in the apoptosis regulatory process. Furthermore, the use of NAC was directed at removing or modulating the route cause of apoptosis-induction i.e., the production and action of reactive nitrogen species. Following treatment of cultured HeLa cells with sodium nitroprusside in the presence of NAC, at a dose that blocks apoptosis (20 mM), total cellular RNA was isolated and examined for gene expression changes using microarray. Significant transcription of mRNA was observed. In one experiment, using Incyte human LifeGrid filters, as many as 43 genes increased or decreased by greater than 2-fold at both 4 and 6 hours following treatment with sodium nitroprusside were blocked by the addition of NAC. As many as 209 genes were increased or decreased by greater than 2-fold at 6 hours following treatment with sodium nitroprusside were blocked by the addition of NAC. Many of these regulated genes were ESTs (e.g., 51 at 6 hours post-treatment) with little or no known biological function annotation. Changes in early' gene expression included classes of genes regulated by NO (reactive nitrogen species (RNS)) and/or by hypoxic stress and include classes of gene known to be associated with cell defense mechanisms to apoptosis and ROS/RNS induced stress. Of those genes regulated as early as 4 and 6 hours post-treatment with sodium nitroprusside, some include genes that were known to be transcriptionally regulated by NO and/or RNS. Also included were genes that were known to be transcriptionally regulated by hypoxic stress which was well characterized as involving ROS and RNS.(72 pages)

L7 ANSWER 16 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-12753 BIOTECHDS

TITLE: Polynucleotide sequences encoding human secretory proteins useful for gene therapy of e.g. genetic deficiency disorders, cancers, and diseases caused by intracellular parasites; recombinant protein gene production via plasmid expression in host cell, sense, antisense, agonist, antagonist, transgenic animal, antibody, cell culture, DNA array and polymerase chain reaction useful in **disease** gene therapy and drug screening

AUTHOR: STUART J; LINCOLN S E; ALTUS C M; DUFOUR G E; CHALUP M S; HILLMAN J L; JONES A L; YU J Y; WRIGHT R J; GIETZEN D; LIU T F; YAP P E; DAHL C R; MOMIYAMA M G; BRADLEY D L; ROHATGI S D; HARRIS B; ROSEBERRY A M; GERSTIN E H; PERALTA C H; DAVID M H; PANZER S R; FLORES V; DAFFO A; MARWAHA R; CHEN A J; CHANG S C; AU A P; INMAN R R

PATENT ASSIGNEE: INCYTE GENOMICS INC

PATENT INFO: WO 2002020756 14 Mar 2002

APPLICATION INFO: WO 2000-US27297 5 Sep 2000

PRIORITY INFO: US 2000-231832 7 Sep 2000

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2002-315658 [35]

AN 2002-12753 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - An isolated human polynucleotide comprising: (a) a polynucleotide sequence (I) comprising one of 184 fully defined sequences given in the specification (S1-184); (b) a naturally occurring polynucleotide sequence (II) at least 90% identical to (S1-184); (c) a polynucleotide (III) complementary to (I); (d) a polynucleotide (IV) complementary to (II); and (e) an RNA equivalent (V) of (I)-(IV).

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a composition (VI) for detecting the expression of secretory polynucleotides comprising at least one of (I-IV) an a detectable label; (**2**) a polynucleotide (V) comprising at least 30 contiguous nucleotides of the sequence (I-IV); (3) detecting (M1) a **target** polynucleotide (TP) in a sample, where the **target** polynucleotide has a sequence (I-IV); (4) detecting (M2) a TP in a sample, where the TP has a sequence (I-IV); (5) a recombinant polynucleotide (VI) comprising a promoter sequence operably linked to a polynucleotide (I-IV); (6) a cell (VII) transformed with (VI); (7) a transgenic organism (VIII) comprising (VII); (8) producing (M3) a secretory polypeptide (IX) encoded by a polynucleotide (I-IV); (9) an isolated secretory polypeptide (SPTM) (X) encoded by at least one of (I-IV); (10) screening (M4) for a test compound that specifically binds (X); (11) a microarray (XI) where at least one element is (V); (12) generating (M5) a transcript image of a sample that contains polynucleotides; (13) screening (M6) a compound for effectiveness in altering the expression of a TP, where the TP comprises a sequence (I-IV); (14) assessing (M7) the toxicity of a test compound (TC); (15) an array (XII) comprising different nucleotide molecules affixed in distinct physical locations on a solid substrate , where at least on of the nucleotide molecules comprises a first oligonucleotide or polynucleotide specifically hybridizable with at least 30 contiguous nucleotides of a TP, which is (I-IV); (16) an isolated polypeptide (XIII), comprising: (a) a polypeptide (XIV) comprising one of 174 fully defined amino acid sequences given in the specification (S185-369); (b) a naturally occurring polypeptide sequence (XV) at least 90% identical to (S185-369); (c) a biologically active fragment (XVI) of a polypeptide with a sequence (S185-369); and (d) an immunogenic fragment (XVII) of a polypeptide with a sequence (S185-369); (17) a polypeptide (XVIII) with a sequence of (S185-369); (18) a polynucleotide (XIX) encoding (XIV-XVII); (19) a polynucleotide (XX) encoding (XIX); (20) an isolated antibody (XXI) which specifically binds to a secretory polypeptide (XIV-XVII); (21) a diagnostic test (M8) for a condition or **disease** associated with the expression of SPTM in a biological sample; (22) a composition (XXII)

comprising (XXI), or labeled (XXI), (XXIII); (23) diagnosing (M9) a condition or **disease** associated with the expression of SPTM comprising administering (XXII) or (XXIII); (24) preparing (M10) a polyclonal antibody with the specificity of (XXI); (25) an antibody (XXIV) produced by (M); (26) a composition (XXV) comprising (XXIV); (27) making (M11) a monoclonal antibody (XXVI) with the specificity of (XXI); (28) a monoclonal antibody (XXVII) produced by (M11); (29) a composition (XXVIII) comprising (XXVII); (30) detecting (PP) in a sample (M12); (31) purifying (M13) a polypeptide (PP) from a sample; (32) detecting (M14) a polypeptide with an amino acid sequence of S185-369 (PP) in a sample; (33) a composition (XXIX) comprising (XIV-XVII); (34) screening (M15) a compound for effectiveness as an agonist (XXX) of (XIV-XVII); (35) a composition (XXXI) comprising (XXX); (36) treating a **disease** or condition associated with decreased expression of functional SPTM, comprising administering (XXIX) or (XXXI); (37) screening (M16) a compound for effectiveness as an antagonist (XXXII); (38) a composition (XXXIII) comprising (XXXII) identified by (M16); (39) treating (M17) a **disease** or condition associated with overexpression of functional SPTM, comprising administering (XXXIII); and (40) screening (M18) for a compound that modulates the activity of (XIV-XVII).

BIOTECHNOLOGY - Preparation: The polynucleotides are produced using standard recombinant techniques. Preferred Polynucleotide: (V) preferably comprises at least 60 contiguous nucleotides of (I-IV). The polynucleotides encode secretory polypeptides that contain signal peptides and/or transmembrane domains Preferred Array: The first oligonucleotide or polynucleotide of (XII) is completely complementary to at least 30, preferably at least 60, contiguous nucleotides of TP, and is most preferably completely complementary. The array is preferably a microarray and further comprises the TP hybridized to a nuccleotide molecule comprosing the first oligonucleotide or polynucleotide. A linker joins at least one of the nucleotide molecules to the solid substrate. Each distinct physical location on the substrate contains multiple nucleotide molecules, and each nucleotide molecule at a single location has the same sequence which differ from those at a different distinct physical location. Preferred Antibody: The antibody (XXI) is: (a) a chimeric antibody; (b) a single chain antibody; (c) a Fab fragment; (d) a F(ab')**2** fragment; or (e) a humanized antibody. The antibody is produced by screening a Fab expression library or a recombinant immunoglobin library. Preferred Method: The probe of (M2) preferably comprises at least 30, most preferably at least 60, contiguous nucleotides. The method of (3) comprises: (a) amplifying TP, or its fragment, using polymerase chain reaction (PCR) amplification; and (b) detecting the presence or absence, and amount of, of the amplified TP, or its fragment. The method of (4) comprises: (a) hybridizing the sample with a probe comprising at least 20 contiguous nucleotides comprising a sequence complementary to the TP, where a hybridization complex is formed between the probe and TP, or its fragments; and (b) detecting the presence or absence, and amount, of the hybridization complex. The method of (8) comprises: (a) culturing (VII) under conditions suitable for expression of (IX); and (b) recovering (IX). The method of (10) comprises: (a) combining (X) with at least one test compound; and (b) detecting the binding of (X) to the test compound and therefore identifying a compound that specifically binds to (X). The method of (12) comprises: (a) labeling the polynucleotides of the sample; (b) contacting the elements of (XI) with the labeled polynucleotides of the sample so that a hybridization complex is formed; and (c) quantifying the expression of the polynucleotides in the sample. The method of (13) comprises: (a) exposing a sample comprising the TP to a compound so that the TP is expressed; (b) detecting altered expression of the TP; and (c) comparing the expression of the TP in the presence of varying amounts/ absence of the compound. The method of (14) comprises: (a) treating a biological sample containing nucleic acids with the TC; (b) hybridizing the nucleic acids of the treated biological sample with a probe comprising at least 20 contiguous nucleotides of (I-IV) so that a

hybridization complex (HC) is formed between the probe and TP, where TP has a sequence of (I-IV), or their fragment; (c) quantifying the amount of HC; and (d) comparing the amount of HC in an untreated sample with that in a treated sample, where a difference in the amount of HC in the treated sample is indicative of the toxicity of the TC. The method of (21) comprises: (a) combining the biological sample with (XXI) to form an antibody:polypeptide complex (APC); and (b) detecting the complex, where the presence of the complex correlates with the presence of the polypeptide in the sample. The method of (24) comprises: (a) immunizing an animal with a polypeptide with a sequence S185-369 (PP), or its immunogenic fragment, to elicit an antibody response; (b) isolating the antibodies from the animal; and (c) screening the isolated antibodies with the polypeptide, and identifying a polyclonal antibody which binds specifically to PP. The method of (27) comprises: (a) immunizing an animal with PP; (b) isolating antibody producing cells from the animal; (c) fusing the antibody producing cells with immortilized cells to form monoclonal antibody-producing hybridoma cells; (d) culturing the hybridoma cells; and (e) isolating from the culture monoclonal which binds specifically to PP. The method of (30) comprises: (a) incubating (XXI) with a sample so that the antibody binds the polypeptide; and (b) detecting the specific binding, which indicates the presence of PP. The method of (31) comprises: (a) incubating (XXI) with a sample to produce binding of the antibody with PP; and (b) separating the antibody from the sample and obtaining the purified PP. The method of (32) comprises: (a) exposing a sample comprising the polypeptide (XIV-XVII) to a compound; and (b) detecting agonist activity in the sample. The method of (34) comprises: (a) incubating (XXI) with the sample to produce binding of PP and the antibody; and (b) separating the antibody from the sample and obtaining the purified PP. The method of (37) comprises: (a) exposing a sample comprising (XIV-XVII); and (b) detecting an antagonist in the sample. The method of (40) comprises: (a) combining (XIV-XVII) with at least one TC under conditions that ensure the activity of the polypeptide; (b) assessing the activity of the polypeptide; and (c) comparing the activity of the polypeptide in the presence/absence of the TC, where a change in activity is indicative that the compound modulates the activity of (XIV-XVII).

ACTIVITY - Cytostatic; hemostatic; immunostimulant; virucide; anti-HIV; hepatotropic; antiinflammatory; protazoacide; antifungal. No suitable data given.

MECHANISM OF ACTION - Protein therapy; gene therapy.

USE - (VI) is useful for producing transgenic animals. (XXIX) or (XXXI) are useful for treating a **disease** or condition associated with decreased expression of functional SPTM. (XXXIII) is useful for treating a **disease** or condition associated with overexpression of functional SPTM.(XXII) or (XXIII) are useful for diagnosing a condition or **disease** associated with the expression of SPTM. (XXI) is useful for performing a diagnostic test for conditions or diseases associated with the expression of SPTM (claimed). (I-IV) are useful for somatic or germline gene therapy to: (i) correct a genetic deficiency (e.g. severe combined immunodeficiency (SCID), thalessemias, hemophilia); (ii) express a conditionally lethal gene product (e.g. against cancers); and (iii) express a protein which affords protection against intracellular parasites (e.g. human immunodeficiency virus, hepatitis B/C virus; fungal parasites e.g. candida albicans, and protozoan parasites e.g. Trypanosoma cruzi). (X) is useful for diagnosing cell proliferative disorders (e.g. arteriosclerosis, cirrhosis, hepatitis), cancers (particularly cancer of the adrenal gland, bladder, bone (marrow), brain, breast cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus), immune disorders (e.g. acquired immunodeficiency **disease** (AIDS), allergies,**diabetes** mellitus), complications of cancer, trauma, hematopoietic cancer (e.g. lymphoma, leukemia), neurological disorders (e.g. epilepsy, stroke,

Parkinson's **disease**), motor neuron disorders (e.g. amyotrophic lateral sclerosis), demyelinating diseases (e.g. hereditary ataxis, multiple sclerosis), bacterial and viral meningitis, abscesses, viral central nervous diseases, prion diseases(e.g. kuru, Creutzfeldt-Jacob **disease**), fatal familial insomnia, nutritional and metabolic disorders of the central nervous system, developmental disorders of the central nervous system (e.g. neurofibromitosis, mental retardation), cerebral palsy, neuroskeletal disorders, peripheral nervous system disorders, dermatomyositis and polymyositis, myopathy, myasthenia gravis, and mental disorders (e.g. anxiety, amnesia, Tourette's syndrome). Polynucleotides complementary to SPTMs can be used to inactivate or inhibit therapeutically relevant genes.

ADMINISTRATION - No details given.

EXAMPLE - cDNA was isolated from cDNA libraries constructed with the UNIZAP vector system (Stratagene Cloning Systems, Inc. (Stratagene), La Jolla CA) or SUPERSCRIPT plasmid system (Life Technologies) using standard techniques. The cDNA was size selected (300-100 base pairs) using column chromatography or agarose gel **electrophoresis**. cDNAs were ligated into suitable plasmids e.g. PBLUESCRIPT (Stratagene) and transformed into competent Escherichia coli. Plasmids were recovered from the host cells by in vivo excision using the UNIZAP vector system and purified using e.g. WIZARD minipreps DNA purification system (Promega). The purified plasmids were stored or the DNA was amplified by polymerase chain reaction (PCR) and the concentration of purified DNA quantified fluorometrically. cDNA sequencing reactions were processed using standard methods or high throughput instrumentation e.g. ABI CATALYST 800 thermal cycler (Applied Biosystems). The cDNA was then electrophoretically separated using the MAGABACE 1000 DNA sequencing system (Molecular Dynamics). Component sequences from chromatograms were subjected to PHRED analysis (base-calling program for DNA sequence traces) analysis and assigned a quality score. The sequences that had at least a required quality score were subjected to processing editing pathways to eliminate e.g. vector and linker sequences, sequences smaller than 50 base pairs, repetitive elements. Processed sequences were then assigned to gene bins, each sequence belonging to only one bin. Sequences in each bin were assembled to form consensus sequences. New sequences were added to bins using BLAST (basic local alignment search tool) and CROSSMATCH and sequences with BLAST scores of greater than or equal to 150 were added to the bins. Bins were compared against each other and those with local similarity of at least 82% were combined and reassembled. Assembled templates were also subject to analysis by STITCHER/EXON MAPPER algorithms which analyzed the probabilities of e.g. variants, spliced exons. The bins were subjected to several rounds of the assembly process. Following assembly, the templates were subjected to motif, BLAST, and functional analysis and categorized into protein hierarchies using standard methods. The sequences were further analyzed by translating each template in all three reading frames and searching each translation against the Pfam database of hidden Markov model-based protein families and domains using HMMER software (Washington University School of Medecine, St Louis MO), and by bioinformatics tools. The template sequences were translated to derive the corresponding longest open reading frame and the polypeptide sequences analyzed by querying against the GenBank protein database (GENPEPT (GenBank version 124)). Therefore full length polynucleotide and polypeptide sequences were obtained.(584 pages)

L7 ANSWER 17 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-11771 BIOTECHDS
TITLE: Identifying specifically cleavable peptide, useful for **targeted** drug delivery and developing protease inhibitors, by incubating test compound with peptide-nucleic acid fusion;
metallo protease cleavage site identification and DNA library construction, useful for therapy, diagnosis and

drug delivery
AUTHOR: REIMHOLZ R; PLOEGER F
PATENT ASSIGNEE: XZILLION GMBH and CO KG
PATENT INFO: WO 2002016574 28 Feb 2002
APPLICATION INFO: WO 2000-EP9102 22 Aug 2000
PRIORITY INFO: DE 2000-1041238 22 Aug 2000
DOCUMENT TYPE: Patent
LANGUAGE: German
OTHER SOURCE: WPI: 2002-269356 [31]
AN 2002-11771 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Identifying specific proteolytically cleavable peptide (I) by incubating a library of fusion molecules (II), comprising a peptide (Ia) and nucleic acid (III) that encodes (Ia) with a proteolytically active sample, then isolating cleavage fragments of (II), and (iii) determining the sequence of (III) in the separated (II).

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) similar method in which the library is treated, in parallel, with a proteolytically active reference sample, fragments isolated, a differential nucleic acid library established and sequences determined; (**2**) similar method in which the library is first incubated with a proteolytically active presample, cleavage products separated and the treated library processed by the new method; (3) specifically proteolytically cleavable substances (A) containing a (I); (4) diagnostic **marker** containing (I); and (5) detecting proteases having a specific activity.

ACTIVITY - Antiasthmatic; osteopathic; cytostatic; cerebroprotective; neurological; antiarthritic; pancreatic; hypotensive; antithrombotic; virucide; protozoacide. No details of tests for any of these activities are given.

MECHANISM OF ACTION - Inhibition of protease. No biological data given.

USE - (III) identified by the method are used for production of specifically proteolytically cleavable substances (A). (A), which may include an active ingredient activated by proteolysis, are useful in treatment of asthma, **osteoporosis**, cancer, stroke, neuronal diseases, arthritis, pancreatitis, **hypertension**, thrombosis, viral infections and schistosomiasis. Also contemplated are similar compounds designed to release herbicides, insecticides and fungicides, when cleaved. (I) are useful (i) as diagnostic **markers** for specific proteases; (ii) to identify protease inhibitors and (iii), by modification of the peptide sequence, to prepare protease inhibitors.

ADVANTAGE - (I) provide **targeted** delivery of active agents attached to them, so that side effects (and total dose) of the agent are reduced. The method allows protease activity of a sample to be described phenomenologically, without precise knowledge of its active components, and changes in protease activity induced by extracellular factors can be determined, e.g. differential detection of (I) in extracts of healthy and diseased tissues.

EXAMPLE - Fusion molecules were constructed comprising (i) a peptide that contained a cleavage site for matrix metalloprotease-3 (MMP-3) and (ii) the nucleic acid sequence that encoded (i), linked through puromycin. The product was incubated with MMP-3 at pH 7 and 37degreesC for 45 min, then fragments separated by **electrophoresis** to show that cleavage of the fusion molecule had occurred. (37 pages)

L7 ANSWER 18 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-09895 BIOTECHDS
TITLE: Novel antisense compound useful for treating type **2** **diabetes**, cancer and **obesity**, is **targeted** to nucleic acid encoding human protein phosphatase 1B, and hybridizes and inhibits PTP1B expression; useful for gene therapy and functional genomics
AUTHOR: COWSERT L M; WYATT J; FREIER S M; MONIA B P; BUTLER M M;

MCKAY R
PATENT ASSIGNEE: ISIS PHARM INC
PATENT INFO: WO 2002010378 7 Feb 2002
APPLICATION INFO: WO 2000-US23874 31 Jul 2000
PRIORITY INFO: US 2000-629644 31 Jul 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-180079 [23]
AN 2002-09895 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - A compound (Ia) of 8-50 nucleobases in length **targeted** to a nucleic acid molecule (II) encoding protein phosphatase 1B (PTP1B), which specifically hybridizes with and inhibits the expression of PTP1B, or a compound (Ib) of 8-50 nucleobases in length which specifically hybridizes with an 8 nucleobase portion of an active site on (II), is new.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a composition (C) comprising (Ia).

BIOTECHNOLOGY - Preferred Compound: (I) is an antisense chimeric oligonucleotide and comprises at least one modified internucleoside linkage e.g. phosphorothioate linkage. (I) comprises at least one modified sugar moiety e.g. **2**'-O-methoxyethyl sugar moiety, or a modified nucleobase e.g. 5-methylcytosine. Preferred Composition: (C) further comprises a colloidal dispersion system.

ACTIVITY - Antidiabetic; Cytostatic; Anorectic. No biological data is given.

MECHANISM OF ACTION - PTP1B expression inhibitor (claimed); antisense gene therapy. An antisense compound comprising a sequence CTCGGCCCACTGCGCCGTCT or CATGACGGGCCAGGGCGGCT, was analyzed for its effect on human PTP1B mRNA levels by quantitative real-time polymerase chain reaction (PCR). The results showed that the compound demonstrated at least 35 % inhibition of PTP1B.

USE - (I) is useful for inhibiting the expression of PTP1B in liver, kidney or adipose cells or tissues, and for treating an animal preferably human having a **disease** or condition associated with PTP1B, including metabolic **disease** or condition, e.g. type **2** **diabetes**, **obesity** or hyperproliferative condition such as cancer. (I) is also useful for decreasing blood (serum or plasma) glucose levels in an animal e.g. diabetic human or rodent, for preventing or delaying the onset of a **disease** or condition associated with PTP1B in an animal, and preventing or delaying the onset of an increase in blood glucose levels in an animal. (All claimed). (I) is useful as diagnostic and research reagent. (I) is also useful for distinguishing functions of various members of a biological pathway. (I) is useful in antisense gene therapy.

ADMINISTRATION - (II) is administered through topical, pulmonary, intratracheal, intranasal, epidermal, transdermal, oral or parenteral route. Dosage is 0.01 micro-g-100 g/kg.

EXAMPLE - Chimeric oligonucleotides having **2**'-O-alkyl phosphorothioate and **2**'-deoxy phosphorothioate oligo-nucleotide segments were synthesized using an Applied Biosystems automated DNA synthesizer Model 380B. Oligonucleotides were synthesized using the automated synthesizer and **2**'-deoxy-5'-dimethoxytrityl-3'-O-phosphoramidite for the DNA portion and 5'-dimethoxytrityl-**2**'-O-methyl-3'-O-phosphoramidite for 5' and 3' wings. The standard synthesis cycle was modified by increasing the wait step after the delivery of tetrazole and base to 600 s repeated four times for RNA and twice for **2**'-O-methyl. The fully protected oligonucleotide was cleaved from the support and the phosphate group was deprotected in 3:1 ammonia/ethanol at room temperature overnight then lyophilized to dryness. Treatment in methanolic ammonia for 24 hours at room temperature was then done to deprotect all bases and sample was again lyophilized to dryness. The pellet was resuspended in 1M TBAF (undefined) in tetrahydrofuran (THF) for 24 hours at room temperature to deprotect the

2' positions. The reaction was then quenched with 1 M TEAA (undefined) and the sample was then reduced to 1/2 volume before being desalted on a G25 size exclusion column. The oligo recovered was then analyzed spectrophotometrically for yield and for purity by capillary **electrophoresis** and by mass spectrometry. (142 pages)

L7 ANSWER 19 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-08776 BIOTECHDS
TITLE: Set of novel map-related biallelic **markers**, preferably located on **obesity** disorder-associated chromosomal regions on chromosomes 3, 10 and 19, useful, for e.g. detecting statistical correlations between **marker** allele and a phenotype; DNA array, bioinformatic software and bioinformatic hardware for DNA analysis and pharmacogenomics
AUTHOR: COHEN D; BLUMENFELD M; CHUMAKOV I; ABDERRAHIM H; BIHAIN B
PATENT ASSIGNEE: GENSET
PATENT INFO: WO 2002006525 24 Jan 2002
APPLICATION INFO: WO 2000-IB1477 18 Jul 2000
PRIORITY INFO: US 2000-219704 18 Jul 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-155043 [20]
AN 2002-08776 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - A set of novel map-related biallelic **markers**, preferably located on **obesity** disorder-associated chromosomal regions on chromosomes 3, 10 and 19, are new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) a method (M1) of genotyping comprising determining the identity of a nucleotide at a map-related biallelic **marker** in a biological sample, where the map-related biallelic **marker** is selected from the 171 nucleotide sequences (N1) defined in the specification, or their complements; (2) a method (M2) of genotyping comprising determining the identity of a nucleotide at a set of biallelic **markers** in a biological sample, and their complements, where the set comprises 10 map-related biallelic **markers** selected from N1, where the biallelic **markers** are selected to have a heterozygosity rate of at least about 0.18, and are separated from one another by an average distance of 10kb to 200kb; (3) a method (M3) of determining the frequency in a population of an allele of a map-related biallelic **marker**, comprising genotyping individuals from the population for the biallelic **marker** according to M1 and determining the proportional representation of the biallelic **marker** in the population; (4) a method (M4) of detecting an association between an allele and a phenotype, comprising determining the frequency of at least one map-related biallelic **marker** allele in a trait positive population according to M3, determining the frequency of the map-related biallelic **marker** allele in a control population according to the M3, and determining whether a statistically significant association exists between the allele and the phenotype; (5) a method (M5) of estimating the frequency of a haplotype for a set of biallelic **markers** in a population, comprising: (a) genotyping each individual in the population for at least one map-related biallelic **marker**; (b) genotyping each individual in the population for a second biallelic **marker** by determining the identity of the nucleotides at the second biallelic **marker** for both copies of the second biallelic **marker** present in the genome; and (c) applying a haplotype determination method to the identities of the nucleotides determined in steps a) and b) to obtain an estimate of the frequency; (6) a method (M6) of detecting an association between a haplotype and a phenotype, comprising estimating the frequency of at least one haplotype in a trait positive population according to M5, estimating the frequency of the haplotype in a control

population according to M5, and determining whether a statistically significant association exists between the haplotype and the phenotype; (7) a method (M7) of identifying a gene associated with a detectable trait comprising: (a) determining the frequency of each allele of at least one map-related biallelic **marker** in individuals having the detectable trait and individuals lacking the detectable trait according to M3; (b) identifying at least one allele of the biallelic **marker** having a statistically significant association with the detectable trait; and (c) identifying a gene in linkage disequilibrium with the allele; (8) a method (M8) of identifying biallelic **markers** associated with a detectable trait, comprising: (a) determining the frequencies of a set of biallelic **markers** comprising at least one map-related biallelic **marker** selected from N1 in individuals who express the detectable trait and individuals who do not express the detectable trait; and (b) identifying at least one biallelic **marker** in the set which are statistically associated with the expression of the detectable trait; (9) a method (M9) for determining whether an individual is at risk of developing a detectable trait or suffers from a detectable trait associated with the trait comprising obtaining a nucleic acid sample from the individual, screening the nucleic acid sample with at least one map-related biallelic **marker** selected from N1 and determining whether the nucleic acid sample contains at least one biallelic **marker** statistically associated with the detectable trait; (10) a method (M10) of administering a drug or treatment comprising obtaining a nucleic acid sample from an individual, determining the identity of the polymorphic base of at least one map-related biallelic **marker** which is associated with a positive response to the drug or treatment, or at least one map-related biallelic **marker** which is associated with a negative response to the drug or treatment and administering the drug or treatment to the individual if the nucleic acid sample contains at least one biallelic **marker** associated with a positive response to the drug or treatment, or if the nucleic acid sample lacks at least one biallelic **markers** associated with a negative response to the drug or treatment; (11) a method (M11) of selecting an individual for inclusion in a clinical trial of a drug or treatment; (12) a method for comparing a first sequence to a reference sequence, comprising reading the first sequence and the reference sequence through use of a computer program which compares sequences, and determining differences between the first sequence and the reference sequence with the computer program, where the first sequence is the sequence of a polynucleotide comprising a contiguous span of 12 nucleotides selected from N1 comprising a map--related biallelic **marker**; (13) use of a polynucleotide for use in determining the identity of nucleotides at a map-related biallelic **marker** selected from N1, where the determining is performed in a hybridization assay, sequencing assay, microsequencing assay, or an enzyme-based mismatch detection assay; (14) use of a polynucleotide for use in amplifying a segment of nucleotides comprising a map-related biallelic **marker** selected from N1; and (15) use of a computer readable medium or a computer system comprising a processor and a data storage device having stored on it the sequence of a polynucleotide comprising a contiguous span of 12 nucleotides selected from the N1 comprising a map-related biallelic **marker**, to analyze a nucleotide sequence.

WIDER DISCLOSURE - Also disclosed as new are: (1) a map of the human genome or its region comprising an ordered array of biallelic **markers**; (2) isolated, purified or recombinant polynucleotides consisting or comprising contiguous span of nucleotides of a sequence selected from one of the 513 sequences (the first 171 sequences are defined in the specification), or its complements; and (3) isolated nucleic acid molecules that comprise or consist of a sequence at least 90%, preferably 99% identical to the nucleotide sequences described above, or a polynucleotide which hybridizes under stringent hybridization conditions to nucleotide sequences described above.

BIOTECHNOLOGY - Preferred Method: In M1, the map-related biallelic **marker** is selected from the first 162 sequences of N1, or their complements. The identity of a nucleotide at 5 biallelic **markers** selected from the first 100 sequences of N1 or their complements is determined. Alternatively, the identity of a nucleotide at 10, 20, 50 or 100 biallelic **markers** selected from N1 or their complements is determined. In M1, the biological sample is derived from a single subject or multiple subjects. The identity of the nucleotides at the biallelic **marker** is determined for both copies of the biallelic **marker** present in the subject's genome. M1 further comprises amplifying, by polymerase chain reaction (PCR) a portion of the sequence comprising the biallelic **marker** prior to the determining step. The determining is performed by a hybridization assay, a sequencing assay, a microsequencing assay, or an enzyme-based mismatch detection assay. In M2, the set of biallelic **markers** comprises 20 or 100 map-related biallelic **markers** selected from N1, where the biallelic **markers** are selected to have a heterozygosity rate of at least 0.18, and are separated from one another by an average distance of 10kb to 200kb. The map-related biallelic **markers** are selected to have a heterozygosity rate of at least 0.32. The map-related biallelic **markers** are separated from one another by an average distance of 25kb to 50kb. In M3, the map-related biallelic **marker** is selected from the first 162 sequences of N1, or their complements. The genotyping is performed on each individual of the population. The genotyping is performed on a single biological sample derived from the population. In M4, each of the genotyping is performed on a single pooled biological sample derived from each of the populations or is performed separately on biological samples derived from each individual in the populations. In M4, the identity of the nucleotides at 10 of the biallelic **markers** selected from N1 is determined. In M4 and M6, the control population is a trait negative population. The case control population is a random population. The phenotype is selected from **disease**, drug response, drug efficacy, treatment response, treatment efficacy, and drug toxicity. In M5, the haplotype determination method is selected from asymmetric PCR amplification, double PCR amplification of specific alleles, the Clark method, or an expectation maximization algorithm. In M5, the map-related biallelic **marker** is selected from the first 162 sequences of N1, or their complements. M7 further comprises identifying a mutation in gene which is associated with the detectable trait. In M9, the detectable trait is selected from **disease**, drug response, drug efficacy, treatment response, treatment efficacy, and drug toxicity. M11 comprises: (a) obtaining a nucleic acid sample from an individual; (b) determining the identity of the polymorphic base of at least one map-related biallelic **marker** which is associated with a positive response to the drug or treatment, or at least one biallelic **marker** associated with a negative response to the drug or treatment in the nucleic acid sample; and (c) including the individual in the clinical trial if the nucleic acid sample contains at least one biallelic **marker** which is associated with a positive response to the drug or treatment, or if the nucleic acid sample lacks at least one biallelic **markers** associated with a negative response to the drug or treatment. In M11, the administering step comprises administering the drug or treatment to the individual if the nucleic acid sample contains at least one biallelic **marker** associated with a positive response to the drug or treatment, and the nucleic acid sample lacks at least one biallelic **marker** associated with a negative response to the drug or treatment. Preferred Polynucleotide: The polynucleotide is attached to a solid support, preferably an addressable array. The polynucleotide further comprises a label.

USE - The methods are useful for genotyping or estimating the frequency of an allele in a population; for detecting an association between a genotype or haplotype and a phenotype e.g. a **disease** involving drug responses (claimed), **obesity** or disorders

related to **obesity** e.g. hyperuricemia, digestive pathology, hepatic function disorders, cancer, cardiovascular **disease**, **hypertension**, hyperlipidemia, insulin disorders, atheromatous **disease** and cardiac insufficiency. The **markers** are useful for detecting a statistical correlation between a biallelic **marker** allele and a phenotype and/or between a biallelic **marker** haplotype and a phenotype.

EXAMPLE - Donors were unrelated and healthy. They presented a sufficient diversity for being representative of a French heterogeneous population. The DNA from 100 individuals was extracted and tested for the detection of the biallelic **markers**. Thirty microlitres of peripheral venous blood were taken from each donor in the presence of EDTA. Cells pellet were collected after centrifugation for 10 minutes at 2000 rpm. Red cells were lysed by a lysis solution (50 microltres final volume: 10 mM Tris pH 7.6, 5 mM magnesium chloride, 10 mM sodium chloride). The solution was centrifuged (10 minutes, 2000 rpm) as many times as necessary to eliminate the residual red cells present in the supernatant, after resuspension of the pellet in the lysis solution. The pellet of white cells was lysed overnight at 42 degrees Centigrade with 3.7 ml of lysis solution composed of 3 ml Th 10-**2** (Tris-HCl 10 mM, EDTA **2** mM)/NaCl 0.4 M; 200 microlitres SDS 10%; 500 microlitres K-proteinase (**2** mg K-proteinase in TE 10-**2** /NaCl 0.4 M). For the extraction of proteins, 1 ml saturated NaCl (6M) (1/3.5 v/v) was added. After vigorous agitation, the solution was centrifuged for 20 minutes at 10000 rpm. For the precipitation of DNA, **2** to 3 volumes of 100% ethanol were added to the previous supernatant, and the solution was centrifuged for 30 minutes at 2000 rpm. The DNA solution was rinsed three times with 70% ethanol to eliminate salts, and centrifuged for 20 minutes at 2000 rpm. The pellet was dried at 37 degrees Centigrade, and resuspended in 1 ml TE 10-1 or 1 ml water. The DNA concentration was evaluated by measuring the OD at 260 nm (1 unit OD = 50 micrograms/ml DNA). To determine the presence of proteins in the DNA solution, the OD 260 I OD 280 ratio was determined. Only DNA preparations having an OD 260/OD 280 ratio between 1.8 and **2** were used. The pool was constituted by mixing equivalent quantities of DNA from each individual. The amplification of specific genomic sequences of the DNA samples was carried out on the pool of DNA obtained previously using 24 amplification primers defined in the specification. In addition, 50 individual samples were similarly amplified. PCR assays were performed using the protocol: Final volume 25 microlitres; DNA **2** ng/microlitre; Magnesium chloride 2mM; dNTP (each) 200 microMolar; primer (each) **2**.9 ng/microlitre; Ampli Taq Gold DNA polymerase 0.05 unit/microlitre; PCR buffer (10x = 0.1 M TrisHCl, pH 8.3, 0.5 M KCl) 1 x. Pairs of first primers were designed to amplify the promoter region, exons, and 3' end of the candidate asthma-associated gene using the sequence information of the candidate gene and the OSP software (undefined). These first primers were about 20 nucleotides in length and contained a common oligonucleotide tail upstream of the specific bases **targeted** for amplification which was useful for sequencing. The synthesis of these primers was performed following the phosphoramidite method, on a GENSET UFPS 24.1 synthesizer. DNA amplification was performed on a Genius II thermocycler. After heating at 94 degrees Centigrade for 10 mins., 40 cycles were performed. Each cycle comprised: 30 sec at 94 degrees Centigrade, 55 degrees Centigrade for 1 min, and 30 sec at 72 degrees Centigrade. For final elongation, 7 min at 72 degrees Centigrade ended the amplification. The quantities of the amplification products obtained were determined on 96-well microtiter plates, using a fluorometer and Picogreen as intercalant agent (Molecular Probes). The sequencing of the amplified DNA obtained was carried out on ABI 377 sequencers. The sequences of the amplification products were determined using automated dideoxy terminator sequencing reactions with a dye terminator cycle sequencing protocol. The products of the sequencing reactions were run on sequencing gels and the sequences were analyzed. The sequence data were further evaluated using the above mentioned

polymorphism analysis software designed to detect the presence of biallelic **markers** among the pooled amplified fragments. The polymorphism search was based on the presence of superimposed peaks in the **electrophoresis** pattern resulting from different bases occurring at the same position. Allelic frequencies were determined in a population of random blood donors from French Caucasian origin. Their wide range is due to the fact that, besides screening a pool of 100 individuals to generate biallelic **markers** as described above, polymorphism searches were also conducted in an individual testing format for 50 samples. This strategy was chosen here to provide a potential shortcut towards the identification of putative causal mutations in the association studies using them. Biallelic **markers** found in only one individual were not considered in the association studies.(311 pages)

L7 ANSWER 20 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2003-04189 BIOTECHDS
TITLE: Producing a polyunsaturated fatty acid (PUFA), useful in dietary supplements and in treating diseases e.g., cancer, comprises expressing human delta 5-desaturase enzyme and exposing enzyme to substrate PUFA to convert to product PUFA; recombinant enzyme protein production via plasmid expression in host cell for use in producing a polyunsaturated fatty acid for use in therapy
AUTHOR: MUKERJI P; LEONARD A E; HUANG Y; PARKER-BARNES J M
PATENT ASSIGNEE: ABBOTT LAB
PATENT INFO: US 6428990 6 Aug 2002
APPLICATION INFO: US 1999-439261 12 Nov 1999
PRIORITY INFO: US 1999-439261 12 Nov 1999; US 1997-833610 11 Apr 1997
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-730518 [79]
AN 2003-04189 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Producing (M1) a polyunsaturated fatty acid (PUFA), comprises: (i) isolating a fully defined human DELTA5-desaturase gene sequence (I) of 1335 bp as given in the specification; (ii) constructing a vector comprising (I); (iii) introducing the vector into a host cell for expression of the human DELTA5-desaturase enzyme (II); and (iv) exposing (II) to a substrate PUFA (III) such that it is converted to a product PUFA (IV).

BIOTECHNOLOGY - Preferred Method: In (M1), (II) is exposed to (III), which is dihomo-gamma-linolenic acid (DGLA) or 20:4n-3, to convert the substrate to (IV) which is arachidonic acid (AA) or eicosapentaenoic acid (EPA), respectively. (M1) further involves: (i) exposing (IV) to an elongase to convert (IV) to another PUFA (V), preferably adrenic acid or (n-3)-docosapentaenoic acid, respectively, and (ii) exposing (V) to an additional desaturase in order to convert (V) to a final PUFA preferably (n-6)-docosapentaenoic acid or docosahexaenoic (DHA) acid.

ACTIVITY - Tranquilizer; Vasotropic; Antiinflammatory; Antiarthritic; Antirheumatic; Antiasthmatic; Antipsoriatic; Osteopathic; Immunomodulator; Antidiabetic; Cytostatic; Dermatological; Neuroprotective; Gynecological; Anti-HIV. No supporting data provided.

MECHANISM OF ACTION - None provided.

USE - The method is useful for producing a polyunsaturated fatty acid such as arachidonic acid (AA), eicosapentaenoic acid (EPA), adrenic acid, (n-3)-docosapentaenoic acid, (n-6)-docosapentaenoic acid and/or docosahexaenoic (DHA) acid (claimed). The PUFAs produced by (M1), such as arachidonic acid (AA), eicosapentaenoic acid (EPA) and/or docosahexaenoic (DHA) acid, are useful for replicating the PUFA content of human breast milk or to alter the presence of PUFAs normally found in a non-human mammal's milk. PUFAs produced by (M1) may be added to a dietary substitute or supplement, particularly an infant formula, for patients undergoing intravenous feeding or for preventing or treating malnutrition or other conditions or **disease** states. The PUFAs are useful for

producing nutritional compositions e.g., any food or preparation for human consumption including for enteral or parenteral consumption, which when taken into the body serve to nourish or build up tissues or supply energy and/or maintain, restore or support adequate nutritional status or metabolic function. The PUFAs are also useful in animal feed supplements to alter an animal tissue or milk fatty acid composition to one or more desirable for human or animal consumption, in animal feed substitutes, animal vitamins or in animal topical ointments. The PUFAs produced by this method are useful in producing pharmaceutical compositions for treating rough or aging skin, injured or burnt skin, angioplasty, inflammation, rheumatoid arthritis, asthma, psoriasis, **osteoporosis**, kidney or urinary tract stone, cancer, cachexia associated with cancer, **diabetes**, eczema, AIDS, multiple sclerosis. PUFAs are also useful in reducing blood pressure, inhibiting platelet aggregation, inducing vasodilation, reducing cholesterol levels, inhibiting proliferation of fibrous tissue, treating endometriosis, and myalgic encephalomyelitis.

EXAMPLE - Fragment containing the human DELTA-desaturase gene was polymerase chain reaction (PCR) amplified using expand high fidelity PCR system and a set of primers containing appropriate restriction sites. The upstream primer designated RO676 (5'-ATACGTGAATTCGCCGCCACCATGGCCCCCGACCCG GTG-3' corresponded to the sense strand of DELTA5 cDNA and contained an EcoRI site 5' upstream of the ATG. The downstream primer RO677 (5'-TATCCGCTCGAGTTATTGGTGAAGATAGGCATCTAG-3') corresponded to the antisense strand at the 3' end of the DELTA5 cDNA, and included an XhoI site immediately downstream of the translational termination codon. The human DELTA5 PCR amplified product was analyzed by agarose-gel **electrophoresis**, gel purified, digested with EcoRI and XhoI, and then ligated into pFastBac1 baculovirus donor plasmid. The respective baculovirus clone was designated as pJPBh4 for the human DELTA5-desaturase. This pFastBac1 vector contained an expression cassette which had a polyhedrin promoter, a SV40 polyadenylation signal, and a gentamycin resistance **marker**. The initial transformation was done in XL1 blue cells. Positive clones were then transformed into Escherichia coli DH10Bac which contained the baculovirus genome. The positive clones were selected by blue white screening in which white colonies contain the recombinant bacmid. White colonies were then selected for bacmid DNA isolation. DNA was isolated using a Qiagen plasmid isolation kit specific for DNA over 135 kb long. The recombinant bacmid DNA was analyzed on a 0.6% agarose gel. PCR analysis, using pUC/M13 primers forward 5'-TGTAAAACGACGGCCAGT-3' and 5'-GAAACAGCTATGACCATG-3' was also performed to confirm the correct insert size for the desaturase cDNA within the bacmid. The Sf9 insect cell Spodoptera frugiperda were used for the recombinant bacmid DNA transfection. Transfection was carried out according to the cell-FECTIN Sf900 protocol. The recombinant virus was recovered by collecting the supernatant at 72 hours post-transfection. A recombinant viral stock was made for the expression studies. All infections with the recombinant virus were done during the mid-logarithmic growth phase of the Sf9's and infected at 5 multiplicity of infection (MOI). To analyze the activity of the expressed human DELTA5-desaturase gene, the Sf9m cells were plated at a concentration of 1x106 cells/well in a 6-well tissue culture plate and infected with 100 mul of the virus stock. The substrate, dihomo-gamma-linolenic acid (DGLA, C20:3n-6). The medium was collected 48 hours post infection and saved. The cells were collected and submitted for lipid analysis. For fatty acid analysis, cell pellets were vortexed with 6 ml of methanol, followed by the addition of 12 ml of chloroform and tridecanoin (as internal standard). The mixtures were incubated for one hour at room temperature or at 4degreesC overnight. The chloroform layer was extracted and filtered with one gram of anhydrous sodium sulfate to remove particulates and residual water. The organic solvents were evaporated at 40degreesC under a stream of nitrogen. The extracted lipids were derivatized to fatty acid methyl ester (FAME) for gas chromatography analysis (GC). The samples were heated at 95-100degreesC

for 30 minutes and cooled to room temperature. Appromixately **2** ml of the 14% boron trifluoride in methanol was added and the heating repeated. After the extracted lipid mixture cooled, **2** ml of water and 1 ml of hexane were added to extract the FAME for GC analysis. The percent conversion was calculated by dividing the product produced by the sum of (the product produced and the substrate) and then multiplying by 100. The conversion of the added substrate, DGLA (c20:3n-6), to arachidonic acid (AA, 20:4n-6) was monitored. The quantity of arachidonic acid (AA, 20:4n-6) produced by the human DELTA5-desaturase was 9.67% of the total fatty acid versus the control which did not produce any AA. This resulted in a 29.6% conversion of DGLA to AA. (104 pages)

L7 ANSWER 21 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2003-01115 BIOTECHDS
TITLE: Novel expression vector comprising DNA segment encoding human zalpha33 polypeptide, operably linked to transcription promoter and terminator, useful for transforming host cells which are used to produce zalpha33 polypeptide; vector-mediated gene transfer and expression in host cell for recombinant protein production, drug screening and gene therapy
AUTHOR: CONKLIN D C; GAO Z
PATENT ASSIGNEE: ZYMOGENETICS INC
PATENT INFO: US 6406888 18 Jun 2002
APPLICATION INFO: US 2000-593995 14 Jun 2000
PRIORITY INFO: US 2000-593995 14 Jun 2000; US 1999-139121 14 Jun 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-616505 [66]
AN 2003-01115 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - An expression vector (I) comprising the following operably linked elements: a transcription promoter; a DNA segment encoding a polypeptide comprising residues 18-178 of a fully defined human zalpha33 (a helical cytokine) polypeptide sequence of 178 amino acids (S2) as given in specification; and a transcription terminator, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a cultured cell (II) into which (I) has been introduced, where the cell expresses the DNA segment; and (**2**) a recombinant zalpha33 polypeptide (III) produced by culturing (II) under suitable conditions.

WIDER DISCLOSURE - The following are disclosed: (1) an isolated human or mouse zalpha33 polypeptide comprising at least 9 contiguous amino acids of (S2) or a fully defined mouse zalpha33 polypeptide sequence of 178 amino acids as given in specification; (**2**) fusion proteins comprising human or mouse zalpha33 polypeptide; (3) an antibody which specifically binds to the human or mouse zalpha33 polypeptide; (4) detecting in a test sample in the presence of antagonist of zalpha33 activity, by using zalpha33 polypeptide; (5) a polynucleotide encoding human or mouse zalpha33 polypeptide, and the degenerate variants of the polynucleotide sequences; (6) variants of human zalpha33 polypeptide; (7) polypeptides having conservative amino acid changes compared with the sequence of (S2); (8) counterpart polypeptides and polynucleotides from other species (orthologs); (9) transgenic mice engineered to express zalpha33 gene and mice that exhibit a complete absence of zalpha33 gene function; and (10) use of zalpha33 polypeptide antagonist for treating cancer, reducing graft rejection, preventing graft-versus-host **disease**, boosting immunity to infectious diseases, treating immunocompromised patients or improving vaccines.

BIOTECHNOLOGY - Preferred Vector: (I) further comprises a secretory signal sequence operably linked to the DNA sequence. Preferably, the signal sequence encodes residues 1-17 of (S2). Preferred Cell: (II) comprises (I) further comprising a secretory signal sequence operably linked to the DNA segment such that the polypeptide is secreted by the

cell.

ACTIVITY - Antirheumatic; Antiarthritic; Neuroprotective; Antiinflammatory; Immunosuppressive; Dermatological; Antiasthmatic; Antibacterial; Antidiabetic; Antiproliferative. No supporting data is given.

MECHANISM OF ACTION - Modulates proliferation, differentiation, migration and metabolism of responsive cell types and regulates tissue development.

USE - (II) is useful for making zalpha33 polypeptide which involves culturing (II) comprising (I) under conditions whereby the DNA segment encoding residues 18-178 of (S2) is expressed and the polypeptide is produced; and recovering the polypeptide. The expression vector further comprises secretory signal sequence operably linked to the DNA segment, such that the polypeptide is secreted by the cell and recovered from the medium in which the cell is cultured (all claimed). (III) is useful for modulating proliferation, differentiation, migration, adhesion, or metabolism of responsive cell types e.g., epithelial cells or cell lines U-373 MG (human brain glioblastoma) and 3A-Sub E (SV40-transformed human placenta). zalpha33 polypeptide has therapeutic applications including autoimmune diseases such as rheumatoid arthritis, multiple sclerosis, myasthenia gravis, systemic lupus erythematosus and **diabetes**. zalpha33 polypeptide regulates inflammation, and thus is useful for treating rheumatoid arthritis, asthma, and sepsis. The polypeptide is also useful for reducing graft rejection, preventing graft-versus-host **disease**, boosting immunity to infectious diseases, treating immunocompromised patients or improving vaccines. zalpha33 polypeptides are also useful as culture medium components for modulating expansion, proliferation, activation, differentiation, migration or metabolism of responsive cell types e.g., hematopoietic cells, endothelial cells, smooth muscle cells, fibroblasts and hepatocytes. The polypeptide is also useful for identifying inhibitors of its activity, and for isolating receptors. The polypeptides are also useful for diagnosis or treatment of disorders associated with cell loss or abnormal cell proliferation. Labeled zalpha33 polypeptides may be used for imaging tumors or sites of abnormal cell proliferation. The proteins are also useful as molecular weight standards, as reagents in assays for determining circulating levels of the protein, such as diagnosis of disorders characterized by over or under production of zalpha33 protein or in the analysis of cell phenotype.

ADMINISTRATION - zalpha33 proteins are administered by topical or parenteral, particularly intravenous or subcutaneous delivery. Dosages for topical application range from 0.1-10 microg/cm2 of the area.

EXAMPLE - An expression plasmid encoding full-length mouse zalpha33 was constructed using the expression vector pEZE2. The vector pEZE2 was derived from pDC312 by the addition of additional restriction enzyme recognition sites to the multiple cloning site. pDC312 and pEZE2 contain an EASE segment, as described in WO 97/25420, which can improve expression of recombinant proteins **two**- to eight-fold in mammalian cells. The pEZE2 expression unit contains the cytomegalovirus (CMV) enhancer/promoter, the adenovirus tripartite leader sequence, a multiple cloning site for insertion of the coding region for the recombinant protein, an encephalomyocarditis virus internal ribosome entry site, a coding segment for mouse dihydrofolate reductase, and the SV-40 transcription terminator. In addition, pEZE2 contains an Escherichia coli origin of replication and a bacterial beta-lactamase gene. A DNA fragment encoding zalpha33 with a C-terminal Glu-Glu tag (zalpha33CEE) was generated by polymerase chain reaction (PCR). The fragment included 5' FseI and 3' AscI sites for direct cloning into the expression vector. The 5' primer contained an FseI site, a Kozak sequence, and the first 31 base pairs of the native leader sequence for zalpha33. The 3' primer contained the last 18 base pairs of zalpha33, a Glu-Glu tag sequence, a stop codon, and an AscI site. The PCR mixture included 1 microl of template (plasmid containing the full-length mouse zalpha33 sequence). The PCR-generated fragment was purified and digested

with AscI and FseI. Five micrograms of the expression vector pEZE2 were also digested with FseI and AscI in a single, 100 microl reaction. The digested DNA was fractionated by agarose gel **electrophoresis**, and the DNA fragments were isolated and purified. Five microliters of the zalpha33CEE DNA fragment and 1 microl of the pEZE2 vector fragment were ligated overnight at room temperature using T4 DNA ligase. One microliter of the ligation mixture was added to 25 microl of electrocompetant E.coli strain DH10B. The mixture was electroporated. To the cuvette, 1 mL of Luria bertani (LB) broth was added, and 100 microl of the mix was plated onto LB/Ampicillin agar plates. The plates were incubated overnight at 37 degrees C, and 16 isolated colonies were picked for DNA mini prep. Individual clones were screened by PCR for the presence of zalpha33CEE DNA, using the above-mentioned primers. DNA sequencing was performed on clone 1-6, to verify the correct full-length sequence. One clone contained the correct expected sequence, from which DNA was prepared using a commercially available kit. The plasmid was designated pKF0248. Plasmid pKF0248 was prepared for transfection into Chinese hamster ovary (CHO) cells. Protein-free and serum-free suspension-adapted CHO DG44 cells were taken from a frozen stock and prepared for transfection by culturing in PFCHO media (undefined), 4 mM L-glutamine and 1X HT supplement at 37 degrees C and 5% CO2 in shake flasks at 120 RPM on a rotating shaker platform. The cells were allowed to recover from the process of thaw before being transfected at passage 38 with the plasmid zalpha33 Mouse-CEE/pEZE2. The CHO DG44 cells were transfected by electroporation while the DNA pellet was drying, and spun in a 25 ml conical centrifuge tube at 900 RPM for 5 minutes. The cells were resuspended into a total volume of 300 microl in PFCHO media and placed in an electroporation cuvette with a 0.4 cm electrode gap. After approximately 50 minutes of drying time the plasmid DNA was resuspended into 500 microl of PFCHO growth media and added to the cuvette. The cuvette was placed in an electroporator and electroporated immediately. The cells were allowed to stand for 5 minutes at room temperature to recover before placement in 20 ml total volume of PFCHO media in a tissue culture flask. Upon recovery from the selection process conditioned media containing the secreted zalpha33 protein is assayed by Western Blot. The protein was detected by chemiluminescence. (41 pages)

L7 ANSWER 22 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-18928 BIOTECHDS
TITLE: Detecting increased risk for developing inflammatory disorder in a mammal, involves detecting a copy of interleukin-1 beta gene haplotype in the mammal comprising cytosine nucleotides at specific positions;
risk assessment for developing inflammatory disorder associated with interleukin-1-beta copy number and pharmacogenetics
AUTHOR: HALL S K; MILOS P M; SEYMOUR A B
PATENT ASSIGNEE: PFIZER PROD INC
PATENT INFO: EP 1217081 26 Jun 2002
APPLICATION INFO: EP 2000-310731 22 Dec 2000
PRIORITY INFO: US 2000-258034 22 Dec 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-550411 [59]
AN 2002-18928 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Detecting (M1) an increased risk of developing an inflammatory disorder, comprising detecting in a sample of DNA taken from a mammal, the presence of at least one copy of an interleukin (IL)-1beta gene haplotype having cytosine nucleotides at positions -31 and +3953 in the mammal, is new. The presence of the copy indicates that the mammal has an increased risk of developing the disorder.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) detecting (M2) a mammalian patient who requires an

increased dosage of an agent that reduces the effect of IL-beta by detecting in a sample comprising DNA taken from the mammal, the presence of at least one copy of an IL-1beta gene haplotype comprising cytosine nucleotides at positions -31 and +3953 in the patient, where the presence of the copy of the IL-1beta gene haplotype indicates that the patient requires a dosage of the agent to reduce the effect of IL-1beta in the patient that is higher than the dosage of the agent required to reduce the effect of IL-1beta in a second mammal who does not have the copy of the IL-1beta haplotype; and (2) a kit (I) for detecting an increased risk of developing an inflammatory disorder in a mammal, comprising: (a) a first set of polymerase chain reaction (PCR) primers for amplifying a first DNA sequence comprising the nucleotide at position -31 of an IL-1beta gene; (b) a second set of PCR primers for amplifying a second DNA sequence comprising the nucleotide at position +3953 of the IL-1beta gene; and(c) instructions for determining the presence of at least one copy of an IL-1beta gene haplotype comprising cytosine nucleotides at positions -31 and +3953 in the mammal based on the size of the PCR products.

BIOTECHNOLOGY - Preferred Method: In M1 or M2, the mammal has **two** copies of the IL-1beta gene haplotype. The IL-1beta gene haplotype further comprises a thymidine nucleotide at position -511. In M2, the mammalian patient and the second mammal are of the same species. Preferred Kit: In (I), the mammal has at least one copy of the IL-1beta gene haplotype comprising cytosine nucleotides at positions -31 and +3953 or has **two** copies of the IL-1beta gene haplotype. (I) further comprises a third set of primers for amplifying a third DNA sequence comprising the nucleotide at position -511 of the IL-1beta gene and instructions for determining the presence of a copy of an IL-1beta gene haplotype comprising cytosine nucleotides at positions -31 and +3953 and a thymidine nucleotide at position -511 in the mammal based on the size of the PCR products.

USE - M1 is useful for detecting an increased risk for developing an inflammatory disorder in a mammal, preferably a human being, where the inflammatory disorder is selected from coronary artery **disease**, **osteoporosis**, nephropathy in **diabetes** mellitus, alopecia areata, Graves' **disease**, systemic lupus erythematosus, lichen sclerosis, ulcerative colitis, periodontal **disease**, juvenile chronic arthritis, chronic iridocyclitis, psoriasis, insulin dependent **diabetes**, diabetic complications, diabetic retinopathy, atherosclerosis, Crohn's **disease**, rheumatoid arthritis, **osteoarthritis**, congestive heart failure, and a neurodegenerative **disease**. M2 is useful for detecting a mammalian patient who requires an increased dosage of an agent that reduces the effect of IL-beta. (I) is useful for detecting an increased risk of developing an inflammatory disorder in a mammal. (All claimed). The methods are useful as analytical or therapeutic tools.

ADVANTAGE - M1 is a reliable method for detecting an increased risk for developing an inflammatory disorder in a mammal.

EXAMPLE - The presence of an interleukin (IL)-1beta haplotype that correlated with an increased occurrence of psoriasis was identified. The genotypes of Caucasian human subjects with psoriasis (and healthy control subjects) was determined. To do this, the following primers flanking the indicated IL-1beta gene polymorphisms were generated. C-511T primers: 5'-TGGCATTGATCTGGTTCATC-3' and 5'-GTTTAGGAATCTTCCCACTT-3', T-31C polymerase chain reaction 1 (PCR1) primers: 5'-CCAATACTCTTTTCCCCTTTCC-3' and 5'-CTTGTGCCTCGAAGAGGTTT-3'. T-31C (PCR2) primers: 5'-CCAATACTCTTTTCCCCTTTCC-3' and 5'-TTCTCCCTCGCTGTTTTCA-3', and C3953T primers: 5'-GTTGTCATCAGACTTTGACC-3', and 5'-TTCAGTTCATATGGACCAGA-3'. PCR amplifications were performed. There were **two** reactions for the T-31C polymorphism. The first reaction amplified a region that contained the polymorphism at position -31. The second reaction used a reverse primer that introduced a G in place of the A three nucleotides downstream of the T-31 C polymorphism. These **two** reactions introduced a NIaIII restriction site that was used to score the genotype, rather than

the naturally occurring AluI restriction site which was a very common restriction endonuclease and thus was difficult to use to interpret the genotypes. To perform the PCR amplifications, 10 ng genomic DNA was amplified in a 50 micro-l reaction containing 10 mM Tris-Cl, pH 8.7, 50 mM KCl, 1.5 mM MgCl2, 200 micro-M each of the dNTPs, 0.5 micro-M each of the PCR primers and **2**.5 Units HotStar Taq DNA polymerase. A second PCR reaction was necessary for the T-31C **marker** since the reverse primer in the reaction introduced an NIaIII site which was more reliable in the restriction fragment length polymorphism (RFLP) assays than the native AluI site. PCR products from the initial PCR reaction were diluted 1:800 and 1 micro-l PCR product was used as template in the second PCR reaction. For genotyping, the PCR products were digested with the appropriate restriction enzyme and buffer. 80-100 ng PCR product was digested with restriction enzyme in a 50 micro-l volume. C-511T samples were digested with AvaI and the T-31C samples were digested with NIaIII at 37 degrees C for 90 minutes. The C3953T samples were digested with TaqI at 65 degrees C for **2** hours. The AvaI and TaqI digests were resolved on **2**.5 % agarose gels. The NIaIII digests were resolved by gel **electrophoresis** on a 3 % agarose gel. Genotypes were scored by visual examination of the size of the PCR products present. The results demonstrated that the patients with psoriasis had at least one copy of an IL-1beta gene haplotype comprising a cytosine nucleotide at position -31 and a cytosine nucleotide at position +3953 as compared to control subjects (i.e. with no psoriasis).(17 pages)

L7 ANSWER 23 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2003-04530 BIOTECHDS
TITLE: Novel isolated neurotransmitter-gated ion channel superfamily member, designated 14691 polypeptide, for treating angiogenesis, cardiovascular, endothelial cell, kidney, neurological, metabolic and immune disorders; recombinant protein production and sense and antisense sequence use in gene therapy
AUTHOR: CURTIS R A J
PATENT ASSIGNEE: MILLENNIUM PHARM INC
PATENT INFO: EP 1245574 2 Oct 2002
APPLICATION INFO: EP 2002-252220 27 Mar 2002
PRIORITY INFO: US 2001-279086 27 Mar 2001; US 2001-279086 27 Mar 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-742714 [81]
AN 2003-04530 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - An isolated neurotransmitter-gated ion channel superfamily member polypeptide (I), designated 14691, encoded by a nucleic acid molecule comprising a 3158 or 2997 nucleotide sequence (S1), given in the specification, a naturally occurring variant of a polypeptide comprising a 998 residue amino acid sequence (S2), given in the specification, or its fragment, is new.

DETAILED DESCRIPTION - An isolated neurotransmitter-gated ion channel superfamily member polypeptide (I), designated 14691, encoded by a nucleic acid molecule comprising a 3158 or 2997 nucleotide sequence (S1), given in the specification, a naturally occurring variant of a polypeptide comprising a 998 residue amino acid sequence (S2), given in the specification, or its fragment, is new. (I) comprises a polypeptide encoded by a nucleic acid molecule comprising S1 or its complement, a naturally occurring allelic variant of S2 encoded by a nucleic acid molecule which hybridizes to S1 or its complement under stringent conditions, or an antigenic fragment of at least 8 contiguous amino acids of S2. INDEPENDENT CLAIMS are also included for the following: (1) an isolated nucleic acid molecule (II) comprising S1, a nucleic acid molecule encoding a polypeptide comprising an antigenic fragment of at least 24 nucleotides of S2, a nucleic acid molecule encoding a

polypeptide comprising S2, a nucleic acid molecule encoding a fragment of at least 15 contiguous amino acid of S2, or a nucleic acid molecule which encodes the naturally occurring allelic variant of S2, where the nucleic acid molecule hybridizes to the nucleic acid molecule comprising S1 or its complement under stringent conditions; (2) a host cell (III) containing (II); (3) an antibody (Ab) which selectively binds (I); (4) producing (I), comprising culturing (III) under expression conditions, and recovering the polypeptide; (5) detecting the presence of (I) in a sample, by using a compound (C1) which selectively binds to (I); (6) detecting the presence of (II) in a sample, by using a nucleic acid probe or primer (P) which selectively hybridizes to (II); (7) a kit (IV) comprising C1 or (P), and instructions for use; (8) identifying a compound which binds to (I); (9) modulating the activity of (I); and (10) identifying a compound which modulates the activity of (I).

WIDER DISCLOSURE - (1) an isolated nucleic acid molecule antisense to (II); (2) nucleic acid constructs or vectors including (II); (3) a **two**-dimensional array having a number of addresses, each having a unique capture probe; (4) molecular beacon oligonucleotide primer and probe molecules; (5) assays for determining a genetic alteration in (I) or (II); (6) analyzing a sample by contacting the sample with the above array and detecting binding of the sample to the array; (7) detectably labeled 14691 probes and primers; (8) 14691 chimeric or fusion proteins; (9) non-human transgenic animals comprising (II), and a population of cells from the transgenic animal; (10) novel agents identified by the above said screening methods; (11) determining if a subject is at a risk for a disorder related to a lesion in or the misexpression of a gene encoding 14691; (12) monitoring the influence of agents (e.g. drugs) on the expression or activity of 14691 protein; (13) analyzing a number of capture probes, and analyzing 14691, e.g. structure, function or relatedness to other nucleic acid or amino acid sequences; (14) a set of oligonucleotides using for identifying single nucleotide polymorphism; (15) a computer readable record of a 14691 sequence that includes recording the sequence on a computer-readable matrix; (16) making the above computer readable record; (17) a medium for holding instructions for performing a method for determining whether the subject has a glutamate receptor-associated or another 14691-associated **disease** or disorder, preferably in an electronic system or in a network; (18) a business method for determining whether the subject has a glutamate receptor-associated or another 14691-associated **disease** or disorder; and (19) an array comprising a 14691 sequence.

BIOTECHNOLOGY - Preparation: (I) is produced by culturing (III) in under conditions in which (II) is expressed (claimed). Preferred Method: The sample for detecting the presence of (I) or (II) comprises mRNA molecules, and is contacted with a nucleic acid probe. Binding of test compound with (I) is detected by direct binding of test compound/polypeptide binding, detection of binding using a competition binding assay and a detection of binding using an assay for 14691-mediated signal transduction. C1 is an antibody. Preferred Sequence: (I) further comprises heterologous amino acid sequences. (II) further comprises vector nucleic acid sequences and a nucleic acid sequence encoding the heterologous polypeptide.

ACTIVITY - Cytostatic; Anti-HIV (human immunodeficiency virus); Cardiant; Antiarteriosclerotic; Vasotropic; Antirheumatic; Antianginal; Hypotensive; Antiarrhythmic; Antidiabetic; Antipsoriatic; Antithyroid; Gynecological; Antiinflammatory; Vulnerary; Nephrotropic; Dermatological; Hemostatic; Antiasthmatic; Neuroleptic; Antidepressant; Tranquilizer; Antiparkinsonian; Antimanic; Antimigraine; Nootropic; Neuroprotective; Analgesic; Metabolic; Anorectic; Ophthalmological; Antiallergic; Antiulcer; Antiarthritic; Osteopathic; Thrombolytic.

MECHANISM OF ACTION - Gene therapy; modulator of expression or activity of 14691 molecules. No biological data is given.

USE - (I) and (II) are useful as diagnostic and therapeutic agents for preventing a **disease** or condition associated with an aberrant or unwanted 14691 activity in a subject, including angiogenesis

(e.g. Kaposi's sarcoma), cardiovascular (e.g. atherosclerosis, ischemic reperfusion injury, angina, **hypertension**, arrhythmia and myocardial infarction), endothelial cell (e.g. diabetic retinopathy, psoriasis, Grave's **disease**, endometriosis and chronic inflammatory **disease**), kidney (e.g. glomerulonephritis, systemic lupus erythematosus, urinary tract infection and amyloidosis), lung (e.g. edema, hemorrhage, bronchial asthma and chronic obstructive pulmonary **disease**), central nervous system (CNS) or neurological (e.g. schizophrenia, depression, anxiety, Parkinson's disorder, mania, bipolar affective disorder, obsessive-compulsive disorder, migraine and Alzheimer's **disease**), pain and metabolic (e.g. anorexia nervosa, cachexia, **obesity** and **diabetes**), and immune (such as Crohn's **disease**, allergic asthma, ulcerative colitis, inflammatory bowel **disease**, rheumatoid arthritis, **osteoarthritis**, multiple sclerosis, dermatitis and thrombocytopenia) disorders. (I) is useful as reagents or **targets** in assays applicable to treatment and diagnosis or 14691-associated disorders. (I), (II) and Ab are useful in screening assays, detection assays (e.g. forensic biology), and predictive medicine (e.g. diagnostic assays, prognostic assays, and monitoring clinical trials and pharmacogenomics). (I) and (II) are useful as query sequences to perform a search against public databases to identify other family members or related sequences. (I) is useful as an immunogen to generate Ab, and as a bait protein in yeast **two**-hybrid or three-hybrid assay to identify other proteins which bind to or interact with 14691. (II) is useful as hybridization probe to identify (II), or as polymerase chain reaction (PCR) primer for the amplification or mutation of (II). (II) is useful in gene therapy, to express (I), to detect 14691 mRNA or a genetic alteration in a 14691 gene, and to modulate 14691 activity. (II) is useful in chromosome mapping, to identify an individual from a minute biological sample (tissue typing), and to aid in forensic identification of the biological sample. Ab is useful to isolate, purify and detect (I), and to diagnostically monitor protein levels in tissue as part of a clinical testing procedure. Fragments of (II) are useful as hybridization probes and primers. (I) and (II) are useful as **markers** of disorders or **disease** states, drug activity and pharmacogenomic profile of a subject. (IV) is useful for producing non-human transgenic animals.

ADMINISTRATION - (I) is administered through parenteral, oral, transdermal, systemic, transmucosal or rectal route, at a dose of 0.001-30 mg/kg, preferably 5-6 mg/kg.

EXAMPLE - Total RNA was prepared from various human tissues by a single step extraction method using RNA STAT-60. Each RNA preparation was treated with DNase at 37 degrees C for 1 hour. Integrity of RNA samples following DNase I treatment was confirmed by agarose gel **electrophoresis** and ethidium bromide staining. After phenol extraction, cDNA was prepared from the sample using the SUPERSCRIPT (RTM) choice system. A negative control of RNA without reverse transcriptase was mock reverse transcribed for each RNA sample. Human 14691 expression was measured by TaqMan (RTM) quantitative polymerase chain reaction (PCR) in cDNA prepared from a variety of normal and diseased (e.g. cancerous) human tissues or cell line. Probes were designed by primer express software based on the sequence of human 14691 gene. Each human 14691 gene probe was labeled using FAM (6-carboxyfluorescein), and the beta2-microglobulin reference probe was labeled with a different fluorescent dye, VIC. Differential labeling of the **target** gene and internal reference gene thus enabled measurement in same well. Forward and reverse primers and the probes for both beta2-microglobulin and **target** gene were added to TaqMan (RTM) Universal PCR master mix. Although the final concentration of primer and probe varied, each was internally consistent within a given experiment. TaqMan matrix experiments were carried out on an ABI PRISM 7700 sequence detection system. The results indicated medium levels of 14691 expression in kidney and normal vein, low levels of 14691 expression in a hemangioma and a

chronic obstructive pulmonary **disease** lung sample. (66 pages)

L7 ANSWER 24 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2003-03153 BIOTECHDS
TITLE: New ABCA12 polypeptide and nucleic acid, useful for manufacturing a medicament for preventing and/or treating diseases resulting from dysfunctions in lipophilic substance transport, e.g. lamellar ichthyosis, congenital cataract; vector-mediated gene transfer, expression in host cell and antibody for recombinant protein production, drug screening and gene therapy
AUTHOR: ARNOULD-REGUIGNE I; PRADES C; NAUDIN L; LEMOINE C; DEAN M; DENEFLE P; ROSIER M
PATENT ASSIGNEE: AVENTIS PHARMA SA; US DEPT HEALTH and HUMAN SERVICES
PATENT INFO: WO 2002064827 22 Aug 2002
APPLICATION INFO: WO 2002-EP1978 12 Feb 2002
PRIORITY INFO: US 2001-267715 12 Feb 2001; US 2001-267715 12 Feb 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-674883 [72]
AN 2003-03153 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - An isolated nucleic acid having a 9112, 8875, 8350 or 8113 base pair sequence (I), given in the specification, or their complements, having at least 8 consecutive nucleotides of (I) or its complement, having at least 80 % nucleotide identity with (I) or its complement, hybridizing under high stringency conditions with (I) or its complement, or encoding a 2595 or 2516 residue amino acid sequence (S2), given in the specification, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) nucleotide probes or primers specific for the ATP-binding cassette transporter A12 (ABCA12) gene, which comprise at least 15 consecutive nucleotides of (I) or its complement, or a sequence having any of the 32 18-30 base pair sequences, given in the specification, or their complements; (**2**) amplifying a region of the nucleic acid; (3) a kit for amplifying the nucleic acid, comprising **2** nucleotide primers whose hybridization position is located, respectively, at 5' and 3' of the region of the nucleic acid, and optionally, reagents necessary for an amplification reaction; (4) detecting the novel nucleic acid; (5) a kit for detecting the nucleic acid, comprising the nucleotide probe or primer of (1), and optionally, reagents necessary for a hybridization reaction; (6) a recombinant vector comprising the novel nucleic acid; (7) a recombinant host cell comprising the recombinant vector of (6) or the novel nucleic acid; (8) an isolated polypeptide comprising (S2), or its fragment, variant or homolog; (9) an antibody directed against the polypeptide of (8); (10) detecting the polypeptide of (8); (11) a diagnostic kit for detecting the polypeptide of (8), comprising the antibody of (9) and a reagent allowing detection of an antigen/antibody complex formed between the polypeptide and the antibody; (12) screening a compound active on the transport of lipid substance, an agonist, or an antagonist of ABCA12 polypeptides; (13) screening an agonist or an antagonist of ABCA12 polypeptides; and (14) an implant comprising the recombinant host cell.

WIDER DISCLOSURE - A method for the production of the ABCA12 polypeptide, or its fragment or variant.

BIOTECHNOLOGY - Preferred Nucleic Acid: The nucleic acid further comprises an 85, 90, 95 or 98 % nucleotide identity with the nucleic acid comprising (I) or its complements. Preferred Probe/Primer: The nucleotide probe or primer comprises a **marker** compound. Preferred Method: Amplifying a region of the nucleic acid comprises contacting the nucleic acid with **2** nucleotide primers, where the first primer hybridizes at position 5' of the region, and the second primer hybridizes at position 3' of the region, in the presence of reagents necessary for an amplification reaction; and detecting the amplified nucleic acid

region. Detecting the nucleic acid comprises contacting the nucleic acid with the above nucleotide probe, and detecting a complex formed between the nucleic acid and the probe. The probe is immobilized on a support. Detecting the polypeptide cited above comprises contacting the polypeptide with the antibody, and detecting an antigen/antibody complex formed between the polypeptide and the antibody. Screening a compound active on the transport of lipid substance, an agonist, or an antagonist of ABCA12 polypeptides comprises: (a) preparing a membrane vesicle comprising ABCA 12 polypeptide having (S2), and a lipid substrate comprising a detectable **marker**; (b) incubating the vesicle obtained in step (a) with an agonist or antagonist candidate compound; (c) qualitatively and/or quantitatively measuring a release of the lipid substrate with the detectable **marker**; and (d) comparing the release of the lipid substrate measured in step (b) with a measurement of a release of a labeled lipid substrate by a membrane vesicle that was not previously incubated with the agonist or antagonist candidate compound. Screening an agonist or an antagonist of ABCA12 polypeptides comprises: (a) incubating a cell that express at least an ABCA12 polypeptide with an anion labeled with a detectable **marker**; (b) washing the cell in step (a), where excess labeled anion that has not penetrated into the cell is removed; (c) incubating the cell in step (b) with an agonist or antagonist candidate compound; (d) measuring the efflux of the labeled anion from the cell; and (e) comparing the efflux of the labeled anion in (d) with that measured with a cell that was not previously incubated with the agonist or antagonist compound. Preferred Vector: The vector is, specifically, an adenovirus. Preferred Antibody: The antibody comprises a detectable compound.

ACTIVITY - Ophthalmological; Antidiabetic; Antilipemic; Antiarteriosclerotic; Antiinflammatory. No biological data is given.

MECHANISM OF ACTION - Gene therapy.

USE - The nucleic acid is useful in detecting polymorphisms and mutations in the ABCA12 gene or in the corresponding protein produced by the allelic form of the ABCA12 gene. The ABCA12 polypeptide, the recombinant vector or host cell are useful in manufacturing a medicament, or in screening an active ingredient, for the prevention and/or treatment of a subject affected by a dysfunction in the lipophilic substance transport or by a pathology located on the chromosome locus 2q34, such as lamellar ichthyosis, polymorphic congenital cataract, or insulin-dependent **diabetes** mellitus. (All claimed). The nucleic acid, polypeptide, the vector and the host cell may also be used in preventing and/or treating cardiovascular diseases, e.g. atherosclerosis, inflammation, or other metabolic diseases.

ADMINISTRATION - Administration can be oral, rectal or parenteral, e.g. intravenous, intradermal or subcutaneous, means. No dosage is given.

EXAMPLE - In a total volume of 11.5 micro-l, 500 ng of mRNA poly(A)+ (Clontech) mixed with 500 ng of oligodT were denatured at 70 degrees C for 10 minutes and then chilled. After addition of 10 units of RNAsin, 10 mM dithiothreitol (DTT), 0.5 mM dNTP, Superscript first strand buffer and 200 units of Superscript II, the reaction was incubated for 45 minutes at 42 degrees C. The poly(A) mRNA from placenta, testis, and fetal brain were used. Polymerase chain reaction (PCR) was performed, and products were analyzed and quantified by agarose gel **electrophoresis**, purified with a P100 column. Human placenta poly(A)+ RNA was used as template to generate the 5' and 3' SMART cDNA libraries. First-amplification primers and nested primers were designed from the cDNA sequence. Amplimers of the nested PCR were cloned. Insert of specific clones were amplified by PCR with universal primers (Rev and -21) and sequenced on both strands. Primers comprising a 27, 25, 30 or 28 base pair sequence, given in the specification, and a 27 or 28 base pair sequence, given in the specification, was used to identify 5' and 3' ends of ABCA12, respectively. Four potential transcripts were identified. Mapping experiments revealed a chromosome locus 2q34 localization. (159 pages)

L7 ANSWER 25 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2003-00748 BIOTECHDS
TITLE: New isolated stem cell growth factor-like polypeptide and polynucleotide, useful in therapeutic, diagnostic or research fields, e.g. inducing differentiation of embryonic and adult stem cells or in treating cancer or hemophilia; vector-mediated gene transfer and expression in host cell for recombinant protein production, drug screening and gene therapy
AUTHOR: NISHIKAWA M; LABAT I; DRMANAC R T; TANG Y T; CHAO C
PATENT ASSIGNEE: KIRIN BEER KK
PATENT INFO: WO 2002051868 4 Jul 2002
APPLICATION INFO: WO 2001-IB2839 25 Jul 2001
PRIORITY INFO: WO 2000-35260 23 Dec 2000; WO 2000-35260 23 Dec 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-575369 [61]
AN 2003-00748 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - An isolated polynucleotide (I) encoding a polypeptide having stem cell growth factor (SCF), is new. (I) comprises a 3095, 1590, 1351, 2797 or 2508 base pair sequence (S1), all given in the specification, its translated protein coding portion, mature protein coding portion, extracellular portion or active domain, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated polynucleotide comprising the complement of (S1); (2) a vector comprising the novel polynucleotide or polynucleotide of (1); (3) a host cell genetically engineered to express the novel polynucleotide, or the polynucleotide of (1); (4) a polypeptide produced by the cell of (3); (5) an antibody specific for the polypeptide of (4); (6) detecting the novel polynucleotide, polynucleotide of (1), or polypeptide of (4) in a sample; (7) identifying a compound that binds to the polypeptide of (4); (8) producing a stem cell growth factor-like polypeptide; (9) a kit comprising the polypeptide; (10) a nucleic acid array comprising the polynucleotide or its unique segment attached to a surface; and (11) treating a subject having need to enhance or inhibit the activity or expression of stem cell growth factor-like polypeptide.

BIOTECHNOLOGY - Isolation: The polynucleotides are isolated from cDNA libraries prepared from mouse femoral bone and human fetal liver, spleen, ovary, adult brain, lung tumor, spinal cord, cervix, ovary, endothelial cells, umbilical cord, placenta, lymphocyte, lung fibroblast, fetal brain or testis. Preferred Polynucleotide: The isolated polynucleotide encoding the polypeptide with biological activity, where the polynucleotide hybridizes to the complement of the polynucleotide cited above under stringent hybridization conditions, or the polynucleotide has greater than 90 % sequence identity with (S1). The polynucleotide is preferably a DNA sequence. Preferred Vector: The vector, preferably an expression vector comprises the polynucleotide. Preferred Host Cell: The polynucleotide is in operative association with a regulatory sequence that controls expression of the polynucleotide in the host cell. Preferred Polypeptide: The polypeptide with a stem cell growth factor-like activity comprises at least 5-10 consecutive amino acids from a 30, 499, 529, 28, 449, 530 or 529 residue amino acid sequence, all given in the specification. Preferred Method: Detecting the polynucleotide in a sample comprises contacting the sample with a compound that binds to and forms a complex with the polynucleotide and detecting the complex to detect the polynucleotide. Alternatively, the method comprises contacting the sample under stringent hybridization conditions with nucleic acid primers that anneal to the polynucleotides, amplifying a product comprising at least a portion of the polynucleotide and detecting the product to detect the polynucleotide in the sample. The polynucleotide comprises an RNA molecule and the method further comprises reverse transcribing an annealed RNA molecule into a cDNA polynucleotide. Detecting the polypeptide in a sample comprises contacting the sample

with a compound that binds to and forms a complex with the polypeptide and detecting the complex to detect the polypeptide. Identifying a compound that binds to the polypeptide comprises contacting the compound with the polypeptide in a cell to form a polypeptide-compound complex, where the complex drives expression of a reporter gene sequence in the cell and detecting the complex by detecting reporter gene sequence expression to identify the compound that binds to the polypeptide. Producing a stem cell growth factor-like polypeptide comprises culturing the host cell to express the polypeptide in the cell and isolating the polypeptide from the cell culture or cells cited above. Treating a subject in need of enhanced activity or expression of stem cell growth factor-like polypeptide comprises administering to the subject a composition of the polypeptide, its agonist, or the polynucleotide encoding the polypeptide in a form and under conditions where the polypeptide is produced, and a carrier. Treating a subject having need to inhibit the activity or expression of stem cell growth factor-like polypeptide comprising administering to the subject a composition of an antagonist to the polypeptide, a polynucleotide that inhibits the expression of the nucleotide sequence encoding the polypeptide, or a polypeptide that competes with the stem cell growth factor-like polypeptide for its ligand, and a carrier. Preferred Array: The nucleic acid array detects full matches or mismatches to the polynucleotide or its unique segment.

ACTIVITY - Nootropic; Neuroprotective; Cytostatic; Virucide; Anti-HIV (human immunodeficiency virus); Antiallergic; Antiinflammatory; Immunosuppressive; Antianemic; Antiasthmatic; Fungicide; Antibacterial; Vulnerary; Dermatological; Hepatotropic; Anticonvulsant; Osteopathic; Antiarthritic; Immunostimulant; Antiulcer; Hemostatic. The immunosuppressive effects of the compositions against rheumatoid arthritis were determined in an experimental animal model system. Killed Mycobacterium tuberculosis in complete Freund's adjuvant was intradermally injected in rats followed by immediately administering the test compound and subsequent treatment every other day until day 24. An overall arthritis score was obtained and analysis revealed that the test compound had a dramatic effect on the swelling of the joints as measured by a decrease of the score.

MECHANISM OF ACTION - Gene Therapy; SCF Agonist; SCF Antagonist.

USE - The polypeptides and polynucleotides are useful in inducing differentiation of embryonic and adult stem cells giving rise to different cell types. They are also useful in diagnosing, treating or preventing allergic reactions, e.g. atopic dermatitis, eczema or asthma, cancers, e.g. leukemia, hemophilia or degenerative diseases like Huntington's **disease** or Alzheimer's **disease**. They may also be used in generating new tissues, e.g. bone marrow, skin, cartilage, tendons, bone, muscle, blood vessels, or neural cells, and organs that may aid patients in need of transplanted tissues. The polynucleotides are used to induce immune response, as hybridization probes, as oligomers or primers for polymerase chain reaction (PCR), or for chromosome and gene mapping. They are also used in diagnostics as expressed sequence tags for identifying expressed genes or for physical mapping of the human genome. The polypeptides are used as molecular weight **markers**, in medical imaging, or as a food supplement. They may be involved in regulation of hematopoiesis and treating myeloid or lymphoid cell disorders, anemia or platelet disorders. They are also involved in wound healing and tissue repair and replacement, and in healing of burns, incisions and ulcers. The polypeptide may also exhibit immune stimulating or immune suppressing activity and is useful in treating immune deficiencies and disorders including severe combined immunodeficiency, caused by genetic, viral, e.g. HIV (human immunodeficiency virus) or hepatitis virus, bacterial or fungal infections such as candidiasis, or from autoimmune disorders e.g. multiple sclerosis, rheumatoid arthritis or graft-versus-host **disease**. The composition is useful for treating **osteoporosis**, **osteoarthritis**, bone degenerative

disorders, or periodontal **disease**, through stimulation of bone and/or cartilage repair or by blocking inflammation or processes of tissue destruction mediated by inflammatory processes.

ADMINISTRATION - Dosage is 0.01-100, preferably 0.1-25 mg/kg. Dosing may be once daily, or equivalent does may be delivered at longer or shorter intervals. Administration of the composition may be oral, rectal, transmucosal, intestinal, parenteral, e.g. intramuscular, subcutaneous, intramedullary injections, intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal or intraocular injections.

EXAMPLE - In order to express soluble stem cell growth factor-like polypeptide, the full-length stem cell growth factor-like DNA was polymerase chain reaction (PCR) amplified from Marathon-ready spleen cDNA library. The primary PCR product was further amplified using nested PCR primers that would generate soluble stem cell growth factor-like polypeptide when expressed in suitable cell lines. The product of the secondary PCR having a 1351 base pair sequence, given in the specification, was cloned in pCDNA3.1/Myc-His (+) A between EcoRI and XhoI sites. The plasmid encoding soluble stem cell factor-like polypeptide and control vectors were transfected into Chinese hamster ovary (CHO) cells using FuGENE-6 transfection reagent. Culture medium, cell lysate and the insoluble cell debris fractions were analyzed be sodium dodecyl sulfate-polyacrylamide gel **electrophoresis** (SDS-PAGE) followed by western blotting with anti-myc antibodies. More than 95 % of the soluble stem cell factor-like polypeptide having a 449 residue amino acid sequence, given in the specification, was found to be secreted and present in the culture medium.(192 pages)

L7 ANSWER 26 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2003-00844 BIOTECHDS
TITLE: Detecting if an organism is homozygous or heterozygous in a **target** sequence, by combining differential hybridization or restriction endonuclease digestion with immobilized array technology or electrophoretic separation; gene mutation detection in plant, mammal or human using DNA primer and DNA probe for **disease** therapy and propagation
AUTHOR: SIEMERING K
PATENT ASSIGNEE: MURDOCH CHILDRENS RES INST
PATENT INFO: WO 2002050305 27 Jun 2002
APPLICATION INFO: WO 2001-AU1643 20 Dec 2001
PRIORITY INFO: AU 2000-2214 20 Dec 2000; AU 2000-2214 20 Dec 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-583551 [62]
AN 2003-00844 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Determining (M) presence or absence of a homozygous or heterozygous change in one or more nucleotides within a **target** nucleotide sequence, using forward and reverse primers which are labeled with reporter molecules which provide separate identifiable signals, or unlabeled primers where detection is accomplished by hybridization of a probe labeled at its 5' and 3' termini, is new.

DETAILED DESCRIPTION - Determining (M) presence or absence of a homozygous or heterozygous change in one or more nucleotides within a **target** nucleotide sequence, comprising: (a) amplifying **target** nucleotide sequence using forward and reverse primers to produce an amplified product, where at least one of the primers is labeled with a reporter molecule capable of facilitating the provision of an identifiable signal which can be distinguished from another receptor molecule if both primers are labeled, and at least one primer and its complementary form comprises a complementary sequence to an oligonucleotide sequence anchored to a solid support, and the primers introduce, abolish or hybridize to a **target** site within the amplified product in the presence or absence of a change in one or more

nucleotides, and subjecting the amplified product to detection unit; or (b) the method of: (i) amplifying the **target** nucleotide sequence using forward and reverse primers to produce an amplified product, where one primer comprises one or more chemically modified nucleotides, bases or phosphodiester bonds so that a nucleotide strand which extends from the primer is resistant to exonuclease activity and the other primer comprises a nucleotide sequences having sense and complementary sequences immobilized to a solid support, where the primers introduces or abolishes a restriction endonuclease site within the amplified product in the presence or absence of a change in one or more nucleotides; (ii) digesting the amplified product with an exonuclease to digest the strand not amplified by the primer comprising the exonuclease-resistant nucleotides, bases or phosphodiester linkages to generate a single-stranded nucleic acid molecule comprising the potential presence or absence of a restriction endonucleases site and a nucleotide sequence complementary to an oligonucleotide sequence immobilized to the solid support; (iii) hybridizing to the single stranded nucleic acid molecule a probe that contains complementary to the restriction site that may have been introduced to generate a partial double-stranded molecule, where the probe comprises **two** reporter molecules capable of facilitating the provision of identifiable signals which can be distinguished from each other; (iv) digesting the partially double-stranded molecule with the restriction endonuclease whose site has been potentially introduced or abolished in the amplified product and subjecting the digested molecule to conditions to permit annealing to a set of the immobilized oligonucleotides comprising oligonucleotides which are sense or complementary to a portion of the amplified sequence introduced by at least one primer; and (v) detecting the relative proportion of signal by the reporter molecules, where an equal proportion of different signals or the presence of only one signal represents a homozygous presence or absence of change in the **target** nucleotide sequence and the presence of a differential signal represents a heterozygous presence or absence of the change in **target** nucleotide sequence. An INDEPENDENT CLAIM is also included for an assay device for determining the presence or absence of a nucleotide or group of nucleotides in a nucleic acid molecule, comprising an array of immobilized oligonucleotides each complementary to a nucleotide sequence within an amplified product digested by one or more restriction endonucleases and unit to screen for the hybridization of a **target** sequence to the immobilized oligonucleotide array.

BIOTECHNOLOGY - Preferred Method: In (Mi), the detection unit comprises detecting the relative proportion of signal or lack of signal by the reporter molecules. The **target** site within the amplified product is a restriction endonuclease site. One or more of the forward or reverse primers introduces a restriction endonuclease site within the amplified product. The solid support is glass or a polymer such as cellulose, nitrocellulose, ceramic material, polyacrylamide, nylon, polystyrene and its derivatives, polyvinylidene difluoride, methacrylate and its derivatives, polyvinylchloride and polypropylene, preferably glass. **Two** or more oligonucleotide sequences are anchored to the solid support in the form of an array. The restriction endonuclease site is recognized by restriction enzymes listed in the specification such as AatI, AatII, AauI, BaeI, BamHI, Cac8I, CbiI, DraIII, DsaI, Eco1301, EcoRI, Esp3I, FspI, GsuI, HphI, ItaI, Ksp22I, LspI, MseI, NarI, PacI, RsaI, SecI, TaqII, UbaDI, Van91I, XagI, Zsp2I, etc. The reporter molecule is chloramphenicol, colorless galactosidase, colorless glucuronide, luciferin, and green fluorescent protein. Alternatively, the differential restriction endonuclease digestion is assessed electrophoretically, where the pattern of electrophoretic separation and/or the pattern of reporter molecule signaling is indicative of homozygous or heterozygous presence or absence of change in the **target** sequence. Preferred Device: The device is packaged for sale and contains instructions for use.

USE - (M) is useful for determining the presence or absence of a

homozygous or heterozygous change in one or more nucleotides within a **target** nucleotide sequence. The **target** sequence is in a eukaryotic cell such as a plant cell or a mammalian cell in particular a human cell, and the **target** sequence is associated with a **disease** condition comprising one or more known genetic mutations. The **disease** condition is adreno-leukodystrophy, atherosclerosis, gaucher **disease**, gyrate atrophy, juvenile onset **diabetes**, **obesity**, paroxysmal nocturnal hemoglobinuria, phenylketonuria, refsum **disease**, tangler **disease** and hemochromatosis conditions involving transporters, channels and pumps such as cystic fibrosis, deafness, diastrophic dysplasia, long-QT syndrome, Menkes syndrome, Pendred syndrome, polycystic kidney **disease**, sickle cell anemia, Wilson's **disease** and Zellweger syndrome, conditions involving signal transduction such as ataxia telangiectasia, baldness, Cockayne syndrome, glaucoma, tuberous sclerosis, Waardenburg syndrome and Werner syndrome, conditions involving the brain such as Alzheimer's **disease**, amyotrophic lateral sclerosis, Angleman syndrome, Charcot-Marie-Tooth **disease**, epilepsy, essential tremor, fragile X syndrome, Friedreich's ataxia, Huntington's **disease**, Niemann-Pick **disease**, Parkinson's **disease**, Prader-Willi syndrome, Rett syndrome, spinocerebellar atrophy and William's syndrome, and conditions involving the skeleton such as Duchenne muscular dystrophy, Ellis-van Creveld syndrome, Marfan syndrome and myotonic dystrophy. (All claimed). (M) is useful for screening for polymorphic variants in the genome of plants such as during the tissue culture stages of plant propagation. The ability to identify polymorphic variants in plants such as due to somaclonal variation prevents unnecessary resources being wasted on plants with undesired properties.

EXAMPLE - A mutation at nucleotide 35 in the connexin 26 gene was identified either in the homozygous or heterozygous state. The mutation was a deletion of a guanine at position 35. This mutation was referred to as 35DELTAG. **Two** primers were developed, each labeled with a different reporter molecule and at least one comprising a nucleotide sequence matching and complementary to oligonucleotide sequences immobilized to a solid support. This sequence on the primer was referred to as a tag sequence. The primers comprised a reporter molecule alone or linked to a tag sequence having matching and complementary sequences immobilized to a solid support. One primer comprised a tag sequence linked to a nucleotide sequence complementary to a region flanking the 35DELTAG region for the forward primer and a region downstream of this location for the reverse primer. The reverse primer introduced a base change in the wild-type sequence thus creating a EcoRII site. If the **target** sequence comprised a 35 DELTAG mutation then the EcoRII site was lost, as EcoRII recognized the nucleotide sequence 5'CCWGG3', where W is A or T. In the connexin 26 gene, the nucleotide sequence recognized by EcoRII was 5'CCTGG3'. A 35 DELTAG mutation removed the G at the 3' position and hence, amplification product from a 35 DELTAG sample did not digest, while the wild-type sequence digested. After amplification and digestion with EcoRII, single-stranded forms of the amplified product were exposed to the immobilized oligonucleotides on the solid support. When the **target** sequence was homozygous wild-type, all the amplification product were digested thus removing the reporter molecule associated with the reverse primer. The complementary immobilized oligonucleotide (+) permitted capture of the tag associated with the forward primer. The matching (sense) immobilized oligonucleotide (-) permitted capture of the sequence complementary to the tag generated by extension of the reverse primer during polymerase chain reaction (PCR). As the reporter molecule associated with the reverse primer was cleaved away by EcoRII digestion, no reporter molecule was detected at the (-) feature of the immobilized oligonucleotide pair, i.e. the ratio of signal from forward to reverse primer was in the order of 1:0. When the 35 DELTAG mutation was present in a homozygous state, there was no digestion of any amplification product and both reporter molecules on

both primers were equally represented, i.e. in a ratio of 1:1. When the 35DELTAG mutation was in the heterozygous state, then the amplification product from the nucleotide sequence carrying the mutation was not cleaved but cleavage occurred in the amplification product from the nucleotide sequence not carrying the mutation. About half of the molecules in the total amplification were cleaved. Accordingly, the ratio of signal from forward to reverse primer was 1:0.5. (73 pages)

L7 ANSWER 27 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-19931 BIOTECHDS
TITLE: Use of genes and their expression profiles associated with osteoblast differentiation for screening modulators bone formation, for diagnosing or treating e.g. **osteoporosis**, or as **markers** for the differentiation process;
expression profiling and drug screening useful for **osteoporosis** gene therapy and diagnosis
AUTHOR: JI D; AXELROD D W; COOK J S; JAISWAL N; EINSTEIN R; HOUGHTON A; MERTZ L
PATENT ASSIGNEE: GENE LOGIC INC; PROCTER and GAMBLE CO
PATENT INFO: WO 2002050301 27 Jun 2002
APPLICATION INFO: WO 2000-US48276 18 Dec 2000
PRIORITY INFO: US 2001-285691 24 Apr 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-557663 [59]
AN 2002-19931 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Genes and their expression profiles are used for: (a) screening modulators of precursor stem cell differentiation into osteoblasts, or bone tissue deposition; (b) diagnosing abnormal deposition of bone tissue, abnormal rate of osteoblast formation or **osteoporosis**; or (c) treating or monitoring treatment of the conditions cited in (b), or monitoring the progression of bone tissue deposition.

DETAILED DESCRIPTION - Genes and their expression profiles, which are associated with precursor stem cells undergoing differentiation into osteoblasts, are used for: (a) screening modulators of precursor stem cell differentiation into osteoblasts, or bone tissue deposition; (b) diagnosing abnormal deposition of bone tissue, abnormal rate of osteoblast formation or **osteoporosis**; or (c) treating or monitoring treatment of the conditions cited in (b), or monitoring the progression of bone tissue deposition. The gene expression profiles are that of genes whose expression patterns are altered when precursor stem cells undergo differentiation into osteoblasts. The gene or gene family member consists of 149 genes deposited with GenBank (RTM). The accession numbers, class, identity and expression profiles of these genes are described in the specification. INDEPENDENT CLAIMS are also included for the following: (1) screening for an agent that modulates the differentiation of precursor stem cells into osteoblasts by comparing the first and second expression profiles of the genes specified, or the activities of the polypeptide encoded by the genes in an osteoblast cell population; (2) diagnosing **osteoporosis**, or a condition characterized by abnormal bone tissue deposition or abnormal rate of osteoblast formation by detecting in a tissue sample the level of expression of and/or activity if a protein encoded by a gene or member of a gene family specified, where differential expression or activity of the gene or gene family member is indicative of the condition; (3) monitoring the treatment of a patient having an abnormal bone deposition, abnormal rate of osteoblasts formation or **osteoporosis** by comparing the patient expression profile or activity to an expression profile or activity from a precursor stem cell population or an osteoblast cell population; (4) treating a patient having an abnormal deposition of bone tissue, abnormal rate of osteoblasts formation or **osteoporosis** by administering a pharmaceutical composition that alters the expression

the gene described above and/or activity of the protein encoded by any of these genes; (5) screening for an agent capable of ameliorating the effects of **osteoporosis** or modulating the deposition of bone tissue by: (a) exposing a cell to the agent; and (b) detecting the expression and/or activity level of the gene(s) or member(s) of a gene family specified; (6) monitoring the progression of bone tissue deposition in a patient by detecting the level of expression and/or activity in a tissue sample of a gene or member of a gene family specified, where differential expression or activity is indicative of bone tissue deposition; (7) a composition comprising **two** oligonucleotides, each of which comprises a sequence that specifically hybridizes to a gene or member of a gene family specified; (8) a solid support where **two** oligonucleotides of (7) are attached; and (9) a computer system comprising: (a) a database containing information identifying the expression and/or activity level in osteoblasts of a set of genes comprising the genes or members of the gene family above; and (b) a user interface to view the information.

BIOTECHNOLOGY - Preferred Expression Profiles: The gene expression profiles comprise the expression levels for a set of genes that are differentially regulated in precursor stem cells compared to osteoblasts. The gene expression profiles or activity level comprises the expression or activity levels, in a cell, of **two** genes or members of the gene family. Preferred Method: In the methods above, the expression and/or activity levels of 2 - 10, preferably all the genes, are detected. In method (1), the agent modulates the level of expression or activity for a gene in the precursor stem cell population to the expression level found in an osteoblast cell population. In method (3), monitoring the treatment of a patient having an abnormal bone deposition, abnormal rate of osteoblasts formation or **osteoporosis** comprises: (a) administering a pharmaceutical composition to the patient; (b) preparing a gene expression profile from a cell or tissue sample from the patient and/or assaying an activity of the protein encoded by at least one gene or member of the gene family; and (c) comparing the patient gene expression profile or activity to a gene expression profile or activity from a precursor stem cell population or an osteoblast cell population. In method (4), treating a patient having an abnormal deposition of bone tissue, abnormal rate of osteoblasts formation or **osteoporosis** comprises: (a) administering a pharmaceutical composition that alters the expression and/or activity of a gene or member of the gene family, or a protein encoded by it; (b) preparing a gene expression profile and/or assaying an activity in a cell or tissue sample of precursor stem cells; and (c) comparing the patient expression profile and/or activity to an expression profile or activity from an untreated precursor stem cell population. Preferred Composition: The composition may comprise 3 - 10 oligonucleotides, each of which comprises a sequence that specifically hybridizes to a gene or a member of the gene family. The oligonucleotides are attached to a solid support, which is a membrane, a glass support, a filter, a tissue culture dish, a polymeric material or a silicon support. Preferred Solid Support: An oligonucleotide is attached covalently or non-covalently to the solid support. The solid support is an array comprising 10 - 10000 different oligonucleotides in discrete locations per square centimeter.

ACTIVITY - Osteopathic. No biological data is given.

MECHANISM OF ACTION - Gene therapy.

USE - The genes and their expression profiles are useful for screening modulators of precursor stem cell differentiation into osteoblasts or bone tissue deposition, or diagnosing, treating and monitoring treatment of abnormal deposition of bone tissue, abnormal rate of osteoblast formation or **osteoporosis**. The methods are useful for treating, diagnosing or identifying the above-mentioned conditions, or monitoring treatment of these conditions (all claimed). Specifically, these conditions include postmenopausal **osteoporosis**, glucocorticoid **osteoporosis** or male **osteoporosis**, osteopenia, osteodystrophy, drug-induced abnormalities in bone formation

or bone loss, conditions that involve altered bone metabolism (e.g. idiopathic juvenile **osteoporosis**), skeletal **disease** linked to breast cancer, mastocytosis, Fanconi syndrome or fibrous dysplasia. The genes and their expression profiles are particularly useful as **markers** for the differentiation process and for identifying therapeutic agents that stimulate bone formation. These are also useful for monitoring **disease** progression, tracking or predicting the progress or efficacy of a treatment regime in a patient, or evaluating the effects of a candidate drug or agent on a cell. A computer system is useful for presenting information that identify the expression level in a tissue or cell of a set of genes comprising **two** of the genes or members of gene families specified (claimed).

EXAMPLE - Human Fetal Stromal Cells (HFSCs) were isolated from the bone marrow of a 20-week human embryo. Total cellular RNA was prepared from the HFSC. Synthesis of cDNA was performed as previously described in WO9705286. This was followed by a polymerase chain reaction (PCR), which consisted of 5 cycles of 94 degrees Centigrade (30 seconds), 55 degrees Centigrade (2 minutes) and 72 degrees Centigrade (60 seconds); followed by 25 cycles of 94 degrees Centigrade (30 minutes), 60 degrees Centigrade (2 minutes) and 72 degrees Centigrade (60 seconds). The PCR products were analyzed on 6 % polyacrylamide sequencing gel. Individual cDNA fragments corresponding to mRNA species were separated by denaturing by polyacrylamide gel **electrophoresis** and visualized by autoradiography. Bands identified as having different expression levels in treated versus untreated HFSC were extracted from the display gel, reamplified, and subcloned into pCR-Script with high efficiency using the PCR-Script cloning kit from Stratagene (RTM). The gene family, which is associated with precursor stem cells undergoing differentiation into osteoblasts, consisted of 149 genes deposited with GenBank (RTM), e.g. Human upstream binding factor, Homo sapiens novel retinal pigment epithelial cell protein, H. sapiens KIAA 1334 protein, H. sapiens CYR61 mRNA, Human mRNA for Nm23 protein, Human novel growth factor receptor, Human Tis11d gene, Human high mobility group protein, Rat serine protease inhibitor 1 mRNA or Rat mRNA for water channel aquaporin 3 (AQP3). (78 pages)

L7 ANSWER 28 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-12901 BIOTECHDS
TITLE: New non-genetic based protein **disease** **markers** for **obesity**, **osteoporosis**, **diabetes**, osteoathritis and **hypertension**, useful in diagnosis and monitoring of treatment for these diseases and to screen for therapeutic compounds; **two**-dimensional **electrophoresis** and antisense oligonucleotide for protein distribution study, drug screening, proteomics analysis and potential gene therapy
AUTHOR: REMBERT P; TAYLOR J; STEINER S; ANDERSON N L; MYERS T
PATENT ASSIGNEE: LARGE SCALE PROTEOMICS CORP
PATENT INFO: WO 2002022165 21 Mar 2002
APPLICATION INFO: WO 2000-US28268 12 Sep 2000
PRIORITY INFO: US 2001-886271 22 Jun 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-362307 [39]
AN 2002-12901 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Non-genetic based protein **disease** **markers** for **obesity**, **osteoporosis**, **diabetes**, osteoathritis and **hypertension**, are new.

DETAILED DESCRIPTION - Non-genetic based protein **disease** **markers** for **obesity**, **osteoporosis**, **diabetes**, osteoathritis and **hypertension**, are new, where **markers** for **obesity** (n=34),

osteoporosis (n=20), **diabetes** (n=9), osteoathritis (n=1) and **hypertension** (n=9) are listed in the specification. INDEPENDENT CLAIMS are also included for the following: (1) determining a **disease** state of a subject suspected of having **obesity**, **osteoporosis**, **diabetes**, osteoathritis or **hypertension** comprising: (a) obtaining a sample containing protein; (b) measuring levels of protein **markers** of the **disease** state, where the **markers** are given in the specification; and (c) comparing with levels in controls from **disease**-free subjects/control standards; (2) binding reagents specific for the proteins, optionally bound to a detectable label; (3) a standardized **two**-dimensional electrophoretic protein distribution from a sample (optionally human serum) from a subject having **obesity**, **osteoporosis**, **diabetes**, osteoathritis or **hypertension** (and optionally being treated with pharmaceuticals); (4) protein **markers** comprising a composition of **two** or more proteins which individually do not have significantly different levels between **disease**/control samples in a method as in (1), but produce a combined value which is significantly different, and methods and binding reagents as in (1) and (2) relating to the **markers**; (5) protein submarkers not altered statistically significantly in the method as in (1) but altered in tandem/opposite in level and direction to protein **markers**, and methods and binding reagents as in (1) and (2) relating to the **markers**; (6) generating an index **marker** for a particular physiological state comprising: (a) determining protein **markers** that differ between samples from a subject with a **disease** state and a control sample; (b) selecting **two** or more of the **markers**; (c) combining the values for the **markers** and determining where the combination of values is altered in a manner of greater statistical significance; (7) index **markers** comprising **two** or more protein **markers** determined by (6); (8) cloning a gene encoding a protein **marker** comprising: (a) determining a partial amino acid sequence of the protein; (b) deducing a nucleotide sequence for a gene encoding the protein; and (c) isolating or synthesizing a gene encoding the nucleotide sequence; and (9) polynucleotides encoding the proteins, and antisense sequences inhibiting gene expression.

BIOTECHNOLOGY - Preferred Proteins: The proteins are preferably isolated. Preparation: The protein **markers** may be detected by: (i) measuring levels of individual proteins in a proteome (i.e. a large number of proteins representing the total relevant portion and preferably all detectable proteins using a particular technique e.g. **two**-dimensional **electrophoresis**) of a sample; (ii) comparing with levels in the proteome of a control subject/control standard; and (iii) detecting if proteins are significantly (preferably p less than 0.001) increased/decreased. The proteins may be prepared by standard recombinant techniques. Preferred Methods: Methods of using the protein **markers** are described in the specification as follows: (a) to screen compounds for biological activity against **obesity**, **osteoporosis**, **diabetes**, osteoathritis or **hypertension** comprising contacting a candidate compound with a subject having one of the **disease**, measuring the level of the protein **marker**, and comparing the level of protein **marker** to the level of the **marker** in a control sample from a subject not having the **disease** state or a control standard; (b) to screen compounds for detection/therapeutic activity against a **disease** state comprising contacting a candidate compound with a protein **marker**, measuring the activity of the **marker** or the binding of the compound to the **marker**, and selecting for further development, compounds that affect activity or bind; (c) to identify biological pathways involved in a **disease** state comprising: (a) obtaining a biological sample from a subject having **obesity**, **osteoporosis**, **diabetes**,

osteoathritis or **hypertension**; (b) determining levels of proteins in the proteome in the sample; (c) comparing the levels of each protein in the proteome to levels of protein in a control sample from a subject not having the **disease** state or a control standard; (d) determining which proteins have statistically higher or lower levels in each sample; (e) identifying several of the determined proteins; and (f) deducing which biological pathways are affected based on the identities of the proteins, where the biological pathways contain a protein having a statistically significant higher or lower level in a comparison between the **2** samples; and (d) to determine whether the effects of **two** agents are cumulative or synergistic comprising: (a) exposing a subject to a first agent and obtaining a protein containing biological sample; (b) exposing a subject to a second agent and obtaining a protein containing biological sample; (c) exposing a subject to a first and second agent and obtaining a biological sample; (d) measuring the levels of protein **markers** in each sample; (e) comparing the changes in levels of protein **markers** between a subject exposed to a first agent, a subject exposed to a second agent, and a subject exposed to a first and second agent; and (f) determining whether the effects of the first agent and second agent are cumulative or synergistic.

ACTIVITY - Anorectic; osteopathic; antidiabetic; antiarthritic; hypotensive. No biological data is given.

MECHANISM OF ACTION - None given.

USE - The **markers** and a new method are useful to diagnose **obesity**, **osteoporosis**, **diabetes**, osteoathritis or **hypertension** in individuals. **Marker** levels may also be used to determine **disease** severity. The **markers** and method can also be used to monitor the efficacy of therapy for the conditions, by comparing **marker** levels between samples from a subject taken at different times. The **markers** identified may also be drug development **targets** for the diseases. The protein **markers** can be used to screen compounds for biological activity against the diseases, which may be included with a carrier in pharmaceutical compositions useful to treat the **disease** states. The **markers** are useful to screen candidate compounds for detection of or therapeutic activity against **disease** states, and to identify biological pathways involved in **disease** states. They are also useful to identify synergistic agents which may be included in pharmaceutical compositions (all claimed).

EXAMPLE - Four hundred pairs of monozygotic human twins were screened for phenotypic **disease** states, by measuring quantitive traits of: total fat mass and percent fat (**obesity**), insulin resistance (**diabetes**), spine and total bone mass density (**osteoporosis**), hip joint gap measurement (**osteoarthritis**), and central and radial blood pressure (**hypertension**). Seventy-nine twin pairs (158 subjects) were discordant for a **disease** state, and since twins were genetically identical the differences did not arise from a genetic process. Whole serum samples (25 micrograms for **obesity** and **diabetes** assessments, otherwise 50 microliters) having approximately 70 mg/ml proteins were subjected to proteometric analysis as described in the specification, in which the quantity of protein in a twin's sample was compared to its respective partner (if any) in the respective twin sample. Data were analyzed statistically by conventional methods for determining a correlation between each peturbed protein and **disease** state, and a list of significant **markers** for each respective **disease** state was generated, given in the specification. (63 pages)

L7 ANSWER 29 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-13125 BIOTECHDS
TITLE: New antisense compound which is **targeted** to nucleic acid encoding phosphorylase kinase alpha 1 and inhibits

expression of kinase protein, useful for treating a condition associated with kinase, e.g. **diabetes**;
phosphorylase-kinase-specific antisense oligonucleotide for use in gene therapy of **diabetes**

AUTHOR: MONIA B P; WYATT J R
PATENT ASSIGNEE: ISIS PHARM INC
PATENT INFO: WO 2002020546 14 Mar 2002
APPLICATION INFO: WO 2000-US26608 7 Sep 2000
PRIORITY INFO: US 2000-657452 7 Sep 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-351759 [38]
AN 2002-13125 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - A compound (I) of 8-50 nucleobases in length **targeted** to a nucleic acid molecule (II) encoding phosphorylase kinase alpha-1 (III), and which specifically hybridizes with and inhibits expression of (III), where (I) specifically hybridizes with at least an 8-nucleobase portion of an active site on (II), is new.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a composition (C) comprising (I).

BIOTECHNOLOGY - Preferred Compound: (I) is an antisense chimeric oligonucleotide, comprising at least one modified internucleoside linkage e.g. phosphorothioate linkage. (I) comprises at least one modified sugar moiety e.g. **2**'-O-methoxyethyl sugar moiety, or a modified nucleobase e.g. 5-methylcytosine. Preferred Composition: (C) further comprises a colloidal dispersion system.

ACTIVITY - Antiinflammatory; cytostatic; antimicrobial; antidiabetic.

MECHANISM OF ACTION - Phosphorylase kinase alpha-1 expression inhibitor (claimed); antisense gene therapy. An antisense compound comprising a sequence cggctcctcatggcgacacg or tgatggcacaggatggtctg, was analyzed for its effect on human Phosphorylase kinase alpha-1 mRNA levels by quantitative real-time polymerase chain reaction (PCR). The results showed that the compound demonstrated at least 60% inhibition of human Phosphorylase kinase alpha-1 expression.

USE - (I) is useful for inhibiting the expression of Phosphorylase kinase alpha-1 in cells or tissues, and for treating an animal having a **disease** condition associated with Phosphorylase kinase alpha-1, e.g. a metabolic disorder such as **diabetes** (claimed). (I) is also useful prophylactically, e.g. to prevent or delay infection, inflammation or tumor formation. (I) is also useful as therapeutic, diagnostic and research reagent, for distinguishing functions of various members of a biological pathway, and in antisense gene therapy.

ADMINISTRATION - (I) is administered through oral, rectal, topical, pulmonary, intratracheal, intranasal, epidermal, intrathecal, intraventricular or parenteral route. Dosage is 0.01 microgram-100 g/kg body weight.

EXAMPLE - Chimeric oligonucleotides having **2**'-O-alkyl phosphorothioate and **2**'-deoxy phosphorothioate oligo-nucleotide segments were synthesized using an Applied Biosystems automated DNA synthesizer Model 380B. Oligonucleotides were synthesized using the automated synthesizer and **2**'-deoxy-5'-dimethoxytrityl-3'-O-phosphoramidite for the DNA portion and 5'-dimethoxytrityl-**2**'-O-methyl-3'-O-phosphoramidite for 5' and 3' wings. The standard synthesis cycle was modified by increasing the wait step after the delivery of tetrazole and base to 600 s repeated four times for RNA and twice for **2**'-O-methyl. The fully protected oligonucleotide was cleaved from the support and the phosphate group was deprotected in 3:1 ammonia/ethanol at room temperature overnight then lyophilized to dryness. Treatment in methanolic ammonia for 24 hours at room temperature was then done to deprotect all bases and sample was again lyophilized to dryness. The pellet was resuspended in 1 M TBAF (undefined) in THF (undefined) for 24 hours at room temperature to deprotect the **2**

' positions. The reaction was then quenched with 1 M TEAA (undefined) and the sample was then reduced to 1/2 volume before being desalted on a G25 size exclusion column. The oligo recovered was then analyzed spectrophotometrically for yield and for purity by capillary **electrophoresis** and by mass spectrometry. (2'-O-(2-methoxyethyl)-(2'-deoxy)-(-2'-O-(methoxyethyl)) chimeric phosphorothioate oligonucleotides were obtained. (140 pages)

L7 ANSWER 30 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-12460 BIOTECHDS
TITLE: Generating reference library of restriction fragments from pooled nucleic acids, by using reference population of restriction fragments to compare frequencies of polymorphic sequences between different population pools; restriction fragment reference library generation, single stranded nuclease, and polymerase chain reaction for **disease**-associated gene identification and plant-associated phenotype trait screening
AUTHOR: BRENNER S
PATENT ASSIGNEE: LYNX THERAPEUTICS INC
PATENT INFO: WO 2002016645 28 Feb 2002
APPLICATION INFO: WO 2000-US26115 21 Aug 2000
PRIORITY INFO: US 2000-227058 21 Aug 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-315468 [35]
AN 2002-12460 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Generating (M1) a reference library comprising a mixture of heterologous nucleic acid fragments, is new.

DETAILED DESCRIPTION - Generating a reference library comprising a mixture of heterologous nucleic acid fragments, comprising: (1) digesting pooled nucleic acids comprising first restriction site with a first RE to produce a mixture of RF; (2) forming first population (P1) of ssDNA fragments from a first subpopulation of RF, which comprise a second restriction site which is different from the first restriction site; (3) forming second population (P2) of ssDNA fragments from a second subpopulation of RF, which do not contain second restriction site, and where the first ssDNA fragments have complementary sequences to the second ssDNA fragments from the second subpopulation when the ssDNA fragments are derived from the same RF; (4) hybridizing P1 and P2 of ssDNA fragments to form a first population of duplexes; and (5) treating the first population of duplexes with a single strand dependent nuclease to digest mismatched duplexes in the first population.

BIOTECHNOLOGY - Preferred Method: The method further includes reannealing intact ssDNA from the nuclease digestion to form a second population of duplexes, isolating the second population of duplexes using biotin precipitation to form a reference population of RF, and amplifying the matched duplexes using polymerase chain reaction (PCR). The single strand dependent nuclease is S1, mungbean endonuclease, especially T7 endonuclease. The method involves digesting pooled nucleic acid with RE to produce a mixture of F1 having first cleavage ends and ligating an Exo III resistant linker to the first cleavage ends of F1 to form a first ligation product (LP1). LP1 is digested with a second RE to form a mixture of second RF (F2), some of which comprise a second cleavage end, and is ligated to an Exo III susceptible linker to form a second ligation product (LP2) which includes LP1. The linker comprises a first member of a binding pair. LP2 is digested with Exo III to form a third ligation product (LP3) population comprising ssDNA comprising end sequences corresponding to the Exo III resistant and Exo III susceptible linkers, and double stranded DNA comprising end sequences corresponding to the Exo III resistant linkers. LP3 is denatured and hybridized to form a reannealed LP3 population. The annealed LP3 population is contacted with

a second member of the binding pair to enrich for duplexes which form a reference population of RF and is further contacted with exonuclease I. The Exo III susceptible linker further comprises biotin.

USE - The method is useful for making a reference library of RF from pooled nucleic acids the contain a sequence polymorphism. The reference libraries are heterogeneous mixtures enriched for polymorphic nucleic acid fragments. Such libraries are useful in identifying single or multiple alleles which are associated with different phenotypes, and to compare the frequency of various polymorphisms in a population of interest. Polymorphisms which occur more frequently in one population than another, can be isolated and identified. When used to analyze other populations, a pool of DNA from individuals having a first phenotype is compared to a population which demonstrates a second phenotype. The reference libraries can be used to screen for polymorphic **markers** in close proximity to genes which may be associated with one or more phenotypes or genotypes. Polymorphisms associated with genotypes which show simple Mendelian inheritance, as well as genotypes or phenotypes associated with a complex trait can be detected. Other phenotypes of biological interest which can be screened include common diseases in humans such as cardiovascular diseases, autoimmune diseases, cancer, **diabetes**, schizophrenia, bipolar disorder and other psychiatric disorders. In addition, polymorphisms in other organisms, i.e., plants, associated with phenotypical traits such as **disease** resistance and yield can also be screened. The polymorphic probes from the reference library are useful to compare the frequency of various polymorphisms between different pools of nucleic acids.

ADVANTAGE - The method provides a significant improvement over conventional **marker** associated studies, as no sequence information is required to generate and use the reference libraries.

EXAMPLE - A conventional pUC19 plasmid was modified to create **two** additional Sau 3A sites between the Taq I sites located at base positions 430 and 906 of the plasmid. This newly created plasmid (p0T2S) was then modified further with the addition of a Taq I site between the **two** new Sau 3A sites, to create the plasmid p1T2S. The **two** plasmids were polymorphic at the new Taq 1 site. The **two** plasmids were digested separately with Sau 3A. Single stranded portions of Sau 3A fragments containing Taq I sites were generated using adaptors and primers given in the specification. The Sau 3A digested p1T2S plasmid was filled in with dGTP and then excess of Q adaptors was added in a conventional ligation reaction to form product, which was then digested with Taq I to give three possible products. To this mixture, an excess of M adaptors were added in a conventional ligation reaction to form the three possible products (A), (B), and (C). After ligating M adaptors, the mixture was treated with exonuclease III to render fragments (A) and (B) single stranded. M and Q primers were then added to the reaction mixture and polymerase chain reaction (PCR) was carried out to form product which was then digested with Sau 3A to remove the Q adaptor. The resulting fragment was then treated with T7 gene 6 5'-exonuclease to produce single stranded fragments (F1). Single stranded portions of Sau 3A fragments lacking Taq I sites (Taq fragments) were generated from the plasmid p0T2S. The Sau 3A digested p0T2S was filled in with dGTP and then an excess of N adaptors were added in a conventional ligation reaction to form product, which was then digested with Taq I to give three possible products (D), (E), and (F). The reaction mixture was then treated with T7 gene 6 exonuclease to render all fragments single stranded, except those (D) having **two** N adaptors attached. After treatment with exonuclease I to eliminate single stranded fragments, N primers were added to the reaction mixture and PCR was carried out to enrich the mixture for fragment (D). The resulting fragments were then treated with exonuclease III to produce single stranded fragments (F2). Fragments (F1) and (F2) were annealed and the 3' strands of the resulting duplexes were extended with T4 DNA polymerase to form fragments having primer binding sites for M and N primers. M and N primers were added to the reaction mixture and the fragments were copied

by PCR. The PCR amplicons from the reaction were separated by gel **electrophoresis** and **two** fragments were identified.(82 pages)

L7 ANSWER 31 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-12235 BIOTECHDS
TITLE: Novel isolated SIGLEC (sialic acid-binding Ig-related lectin) protein molecules useful for treating immune system diseases such as asthma, leukemia, allergic rhinitis, psoriasis, conjunctivitis, Crohn's **disease**;
vector-mediated recombinant gene transfer and expression in host cell for autoimmune, leukemia and asthma **disease** gene therapy, diagnosis and prognosis
AUTHOR: LONGPHRE M; CHANG H; WHITNEY G
PATENT ASSIGNEE: BRISTOL-MYERS SQUIBB CO
PATENT INFO: WO 2002008257 31 Jan 2002
APPLICATION INFO: WO 2000-US23082 21 Jul 2000
PRIORITY INFO: US 2000-220139 21 Jul 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-241565 [29]
AN 2002-12235 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - An isolated SIGLEC (sialic acid-binding Ig-related lectin) protein (I) comprising residues Ala14-Ser198 of a 697 residue SIGLEC-BMS-L3-995-**2** amino acid sequence (S13), given in specification, is new. (I) is encoded by a nucleic acid (NA) that hybridizes under stringent conditions to NA that is complementary to Siglec-BMS-L3a, -L3b, -L3c, -L3d, -L3-995-**2**, -L4a, -L5a, -L5b polynucleotide sequences.

DETAILED DESCRIPTION - An isolated SIGLEC (sialic acid-binding Ig-related lectin) protein (I) comprises an amino acid sequence of Ala14-Ser198 of a 697 SIGLEC-BMS-L3-995-**2** amino acid sequence (S13), given in specification, where (I) is encoded by NA (NA1) that hybridizes under stringent conditions to NA that is complementary to the fully defined Siglec-BMS-L3a, Siglec-BMS-L3b, Siglec-BMS-L3c, Siglec-BMS-L3d, Siglec-BMS-L3-995-**2**, Siglec-BMS-L4a, Siglec-BMS-L5a, Siglec-BMS-L5b polynucleotide sequence of 2565, 2594, 2823, 1665, 3024, 1554, 1676 or 1831 (S1-S8) nucleotides, respectively as given in specification. (I) optionally comprises an amino acid sequence that is encoded by NA (NA2) that hybridizes under stringent conditions to Siglec-BMS-L3a, -L3b, -L3c, -L3d, -L3-995-**2**, -L4a, -L5a, -L5b polynucleotide sequences. INDEPENDENT CLAIMS are also included for the following: (1) a peptide fragment (II) of a SIGLEC-BMS-L3c or -L3d protein sequence of 575 (S11) or 430 (S12) amino acids, given in specification, or (S13); (**2**) a mutant SIGLEC BMS protein (III) comprising cytoplasmic domain, where at least one tyrosine in the cytoplasmic domain is substituted with an amino acid such as phenylalanine, leucine, tryptophan, or threonine; (3) an isolated Siglec nucleic acid molecule (IV) comprising a nucleic acid beginning with codon GCC at position +421 and ending at codon TCA at position +594 of a 1554 nucleotide sequence (S5), where the nucleic acid hybridizes, under stringent conditions to a nucleic acid molecule that is complementary to a 2565, 2954, 2823, 1665, 1676, or 1831 (S1)-(S7) nucleotide sequence, all given in the specification; (4) an isolated Siglec nucleic acid molecule (V) that encodes (I); (5) an isolated nucleic acid molecule which is complementary to (IV) or (V); (6) a vector (VI) comprising (IV) or (V); (7) a host-vector system (VII) comprising (VI) in a suitable host cell; (8) preparation of (I) having a SIGLEC-BMS-L3a, -L3b, -L4a, -L5a, -L5b protein sequence of 544 (S9), 622 (S10), 466 (S14), 463 (S15), or 286 (S16) amino acids, respectively, given in specification, or a sequence of (S11)-(S13); (9) a chimeric protein (VIII) comprising (I) or its fragment fused to a heterologous polypeptide; (10) a chimeric protein (IX) comprising an extracellular or cytoplasmic domain of the polypeptide

of (I) fused to a heterologous polypeptide; (11) an antibody or antibody fragment (X) having an antigen binding site, where the antigen binding site specifically recognizes and binds (I); (12) identifying (M1) a test molecule that modulates an immune response induced by Siglec-10 positive cells comprising: (a) contacting Siglec-10 positive cells with the test molecule; and (b) determining if the immune response is modulated; and (13) modulating (M2) immune response induced by Siglec-10 positive cells by contacting Siglec-10 positive cells with a monoclonal antibody directed against Siglec-10 under suitable conditions so that the immune response is modulated.

WIDER DISCLOSURE - (1) Siglec-BMS nucleotide molecules isolated from various mammalian species, including bovine, ovine, porcine, etc; (2) nucleic acid molecules that exhibit sequence identity or similarity with (IV) or (V); (3) nucleic acid molecules corresponding to different allelic forms, polymorphic forms, alternative precursor transcripts, mature transcripts, and differentially spliced transcripts of (IV) or (V); (4) recombinant nucleic acid molecules that are codon usage variants of (IV) of (V); (5) peptide nucleic acids and antisense molecules that react with (IV) or (V); (6) agonists, antagonists or inhibitors of (I); (7) nucleic acid molecules that selectively hybridize to (IV) or (V);(8) fusion genes including (III) or (IV) fused to non-Siglec-BMS gene sequence; (9) proteins having sequence variations from (I) having sequence of (S1)-(S8); (10) chemically modified derivative protein molecules; (11) anti-idiotypic antibodies raised against (X); (12) kits comprising pharmaceutical compositions therapeutic for immune system diseases such as asthma, leukemia, and other allergic or inflammatory diseases which comprises (I), (IV) or (V), etc; and (13) nucleic acid molecules complementary to (IV) or (V).

BIOTECHNOLOGY - Preparation: Preparing (I) having a (S9)-(S15) or (S16), involves culturing (VII) so as to produce the protein which is then recovered (claimed). Preferred Protein: (I) encoded by NA1 comprises a (S11), (S12) or (S13). (I) encoded by NA2 comprises an amino acid sequence of any one of (S9)-(S15). Preferred Fragment: (II) comprises an amino acid sequence of Ala141-Ser198 of (S13), or comprises a cytoplasmic domain having an amino acid sequence of Lys576-Gln697 of (S13) or its fragment. Preferred Mutant Protein: (III) having an amino acid sequence of (S13) comprises tyrosine in the cytoplasmic domain of any one of the tyrosines at position 597, 641, 667 or 691. Preferred Nucleic Acid: (V) comprises a sequence of: (a) (S1) beginning at codon GGC at position +12 and ending at codon CCA at position +1760; (b) (S2) beginning at codon GAT at position +3 and ending at codon CAA at position +1868; (c) (S3) beginning at codon GGA at position +12 and ending at codon CAA at position +1736; (d) (S4) beginning at codon CCC at position +2 and ending at codon ATG at position +1291; (e) (S5) beginning at codon ATG at position +1 and ending at codon CAA at position +2091; (f) (S6) beginning at codon CTG at position +1 and ending at codon GGC at position +1398; (g) (S7) beginning at codon ATG at position +43 and ending at codon AGA at position +1431; or (h) beginning at codon ATG at position +57 and ending at codon AGT at position +914. Preferred Protein: (IX) comprises Glutathione-S-transferase or immunoglobulin constant region as heterologous polypeptide. Preferred Antibody: (X) is a chimeric antibody having a murine antigen binding site and a humanized region that regulates effector functions. Preferred Method: In (M1), Siglec-10 positive cells are contacted with a test molecule that modulates immune response by **targeting** an extracellular domain of Siglec-10 on Siglec-10 positive cells. The extracellular domain encompasses at least one of the Ig like domains of Siglec-10, where the Ig-like domain is an Ig variable or Ig constant domain of Siglec-10. In (M2), the antibody **targets** extracellular domain (Ig-like domain) of Siglec-10.

ACTIVITY - Antiasthmatic; Antiallergic; Antiinflammatory; Cytostatic; Osteopathic; Antipsoriatic; Ophthalmological; Antirheumatic; Antiarthritic. No biological data is given.

MECHANISM OF ACTION - Immune response modulator; SIGLEC-BMS protein activity inhibitor.

USE - Pharmaceutical compositions comprising (I) are useful for treating immune system diseases such as asthma, leukemia or other allergic or inflammatory diseases. Extracellular domain of (I) represent potential **markers** for screening, diagnosis, prognosis, follow-up assays, and imaging methods. (I) is useful as **target** for drugs which inhibit inflammation, tissue damage and remodeling in asthma, and inflammatory diseases such as allergic rhinitis, **osteoarthritis**, Crohn's **disease**, psoriasis, rheumatoid arthritis, conjunctivitis, etc. (I) is also useful for monitoring the course of **disease** or disorders, and for identifying agents that bind with and/or modulate the biological activity of SIGLEC-BMS proteins. (IV) or (V) are useful as nucleic acid probes or primers which are useful in diagnostic assays. The nucleic acid molecules encoding (I) are useful in diagnosis and/or prognosis methods, and to detect the presence and/or amount of Siglec-BMS nucleotide sequences and/or SIGLEC-BMS proteins in biological sample. The nucleic acid molecules encoding (I) are useful in hybridization assays to identify and/or isolate nucleotide sequences related to Siglec-BMS nucleotide sequences. Siglec-BMS nucleic acid probes are useful for screening genomic library to isolate a genomic clone of Siglec gene. Siglec-BMS gene copy number is determined for detecting diseases or disorders associated with Siglec-BMS transcripts or proteins. (X) is useful in diagnostic assays for detecting (I). Therapeutic compositions comprising (X) is useful for treating or preventing conditions associated with the presence or deficiency of (I). (X) may be used to stain the cell surface of SIGLEC-BMS proteins, and for identifying SIGLEC-BMS within damaged or dying cells for detecting the proteins or its fragments. (X) are useful for screening expression libraries in order to obtain proteins related to SIGLEC-BMS proteins, to enrich or purify the proteins from heterologous population of proteins. The SIGLEC-BMS antibodies are also used to detect, sort or isolate cells expressing SIGLEC-BMS proteins and in diagnostic imaging technology.

ADMINISTRATION - Pharmaceutical compositions comprising (I), (IV) or (V) or vectors comprising the nucleic acid molecules encoding (I) are administered by intramuscular, intravenous, intraperitoneal, subcutaneous, oral route, etc. No dosage is given.

EXAMPLE - Nucleic acid molecules having sialic acid-binding Ig-related lectin (Siglec)-BMS nucleotide sequences were obtained by searching a proprietary expressed sequence tag (EST) database. The search identified a nucleotide sequence, Siglec-BMS (-L3a) that is preferentially expressed in eosinophils from an asthmatic patient. Other cDNA clones having the nucleotide sequences of Siglec-BMS (-L3b, -L3c, -L3d, -L4a, -L5a, and -L5b) were obtained by further mining of the same EST database and acquiring the cDNA clones. DNA from individual cDNA clones was isolated. The purified DNA was then cycle sequenced and subsequently separated and detected by **electrophoresis**. The nucleotide sequences fo the Siglec-BMS cDNA clones were analyzed in all 3 open reading frames (ORFs) on both strands to determine the predicted amino acid sequence of the encoded protein. The nucleotide sequence analysis was performed using SeqWeb version 1.1 using the Translate Tool to predict the amino acid sequences, and using the Structure Analysis Tool for predicting the motifs. Several Ig-like domains were identified in all clones which allowed for further similarity analysis using the Pileup Tool in GCG (Unix version 9.1. One additional Ig domain was identified in the L3 clones, based on this similarity analysis. A comparison of the amino acid sequences of each clone suggested that these cDNA clones included sequences that encoded proteins having sequence homology with human CD33 (Siglec-3). These nucleotide sequences were designated Siglec-BMS which had a 2565, 2954, 2833, 1665, 1554, 1676, 1831, or 2208 nucleotide sequence, all given in specification and the protein sequences had a 544, 622, 575, 430, 466, 463, 486, or 779 residue amino acid sequence, given in the specification, and were designated SIGLEC-BMS. Analysis of the expression patterns of Siglec-BMS transcripts in various human tissues using Northern blot techniques showed a 4.4 kbase transcript in human immune tissues, including spleen, lymph node,

and peripheral blood leukocytes (PBL). Lower levels of Siglec-BMS-L3 transcripts were also detectable in human, thymus, bone marrow, and fetal liver. The Siglec-BMS-L3 transcripts were not detected in human non-immune tissues including brain heart, skeletal muscle, colon, kidney, liver, small intestine, placenta or lung. The binding specificity of the extracellular domain of SIGLEC-BMS-L3A and SIGLEC-BMS-L3 fusion was determined using fluorescent activated cell sorting (FACS) analysis. Mixed white blood cell populations and hematopoietic cell lines were obtained to determine the binding specificities of SIGLEC-BMS-L3A and SIGLEC-BMS-L3. The cells and cell lines used in this analysis included the following: cell lines MB, PM, and TJ which are EBV transformed B-cells. B-cell lymphoblastomas Ramos, HSB-**2**, and Raji; Jurkat which is a T-cell lymphoblastoma; HEL which is a erythroblastic leukemia cell line HEL; and monocytic cell lines U973 and HL60 which were obtained from the American Type Culture Collection. The mixed while blood cell populations were analyzed for binding to the SIGLEC-BMS fusion proteins. There was no difference in the binding of SIGLEC BMSL3 and SIGLEC BMSL3 to the cells that were examined by FACS. A small population fo lymphocyte-sized cells and monocyte-sized cells stained positively for both fusion proteins. Double staining with either anti-CD20 (for B-cells), anti-CD-14 for monocytes, anti-CD4 or anti-CD3 (for T-cells) determined that B-cells and monocytes were binding the fusion the fusion protein, but T-cells were not. Similar FACS analyses were performed with cell lines. The HEL (e.g. an erythroblastic leukemia cell line) and Jurkat (e.g. a T-cell line) cell lines did not stain positively for either SIGLEC BMSL3 fusion protein. Additionally, the Epstein-virus Barr (EBV)-transformed B cell lines MB, PM and TJ did not stain positively. The B-cell lines, Ramos, Raji and HSB2, did stain positively. Although some monocyte binding was observed in whole blood, the monocytic cell lines, U973 and HL60, did not exhibit any binding.(209 pages)

L7 ANSWER 32 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-09610 BIOTECHDS
TITLE: Lipid-raft **targeted** derivative of a soluble polypeptide e.g. a soluble complement regulatory molecule for treating disorders involving complement activity and various inflammatory, neurological and immune disorders; vector-mediated gene transfer and expression in host cell for recombinant protein production and **disease** therapy
AUTHOR: ROWLING P J E; SMITH G P; RIDLEY S H
PATENT ASSIGNEE: ADPROTECH LTD
PATENT INFO: WO 2002004638 17 Jan 2002
APPLICATION INFO: WO 2000-GB3034 7 Jul 2000
PRIORITY INFO: GB 2000-16811 7 Jul 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-164646 [21]
AN 2002-09610 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Soluble derivative (I) of a soluble polypeptide has **two** or more heterologous membrane binding elements with low membrane affinity covalently associated with the polypeptide, the elements being capable of interacting with components of cellular or artificial membranes exposed to extracellular fluids and **target** lipid raft components of membrane.

DETAILED DESCRIPTION - Soluble derivative (I) of a soluble polypeptide has **two** or more heterologous membrane binding elements with low membrane affinity covalently associated with the polypeptide, the elements being capable of interacting with components of cellular or artificial membranes exposed to extracellular fluids and **target** lipid raft components of membrane and bind to lipid rafts to localize the polypeptide at lipid rafts. (I) has **two** or more heterologous membrane binding elements with low membrane affinity

covalently associated with the polypeptide, the elements being soluble in aqueous solution and capable of interacting, independently and with thermodynamic additivity, with components of cellular or artificial membranes exposed to extracellular fluids. INDEPENDENT CLAIMS are also included for the following: (1) preparing (I); (2) a polypeptide portion (II) of (I), comprising a soluble peptide linked to a peptide bond to one peptidic membrane binding element which **targets** a lipid raft and/or including a C-terminal cysteine; (3) a DNA polymer (III) encoding (II); (4) a replicable expression vector (IV) which includes (III); (5) a recombinant host cell prepared by transforming a host cell with (IV); and (6) a pharmaceutical composition comprising (I).

BIOTECHNOLOGY - Preparation: (I) is prepared by expressing DNA encoding the polypeptide portion of the derivative in a recombinant host cell and recovering the product and post translationally modifying the polypeptide to chemically introduce membrane binding elements with selectivity for lipid rafts (claimed). Preferred Derivative: The membrane-binding elements of (I) interact selectively with components of lipid rafts including one or more of phosphatidylserine, phosphatidylglycerol, glycosphingolipids, cholesterol, glycosylphosphatidylinositol (GPI)-anchored proteins associated with lipid rafts, and other protein components of lipid rafts that may be found normally on the exoplasmic face of the cell. The membrane-binding elements mediate internalization of the polypeptide. The polypeptide is a soluble complement regulatory molecule, including but not restricted to CD59 and decay accelerating factor (DAF), preferably modified CD59 or DAF peptide, **targeted** to lipid rafts and the signaling pathways that are associated with lipid rafts. The modified CD59 or DAF peptide is chosen from APT635, APT2063, APT530 and APT2334, having a sequence of 83, 100, 254 and 271 amino acids, respectively, defined in the specification, or APT070 and APT154. (I) includes a derivatized antibody or its fragment which can provide a surrogate receptor localized at a lipid raft to divert a mediator interacting with a lipid raft receptor or which can neutralize a further component of a raft such as a cofactor required for signaling. (I) includes a derivatized chemical or biological entity that possesses the physical property of fluorescence which enables lipid rafts to be identified and/or monitored, or which is involved in a catalytic process either as an enzyme, enzyme substrate or enzyme inhibitor. The derivatized chemical or biological entity can form a covalent chemical bond with proteins, sugar groups or lipids that are localized in lipid rafts thus permitting the isolation and identification of the raft component. The entity contains photo-, chemo-, or enzyme-activated crosslinking groups.

ACTIVITY - Neuroprotective; Nootropic; Cerebroprotective; Antiparkinsonian; Antiallergic; Antiulcer; Antipsoriatic; Antiasthmatic; Dermatological; Hypotensive; Vasotropic; Antirheumatic; Antiarthritic; Antiinflammatory; Ophthalmological; Immunosuppressive; Antianemic; Nephrotropic; Antiinfertility; Antibacterial; Antiatherosclerotic; Cardiant; Vulnerary.

MECHANISM OF ACTION - Modulates the function of lipid rafts either to affect intracellular signaling or to change extracellular functions mediated through the raft domains (claimed). No supporting biological data is given.

USE - (I) is useful for treating disorders amenable to treatment by a soluble peptide fragment of CD59, DAF or other therapeutic agent, and for the preparation of a medicament for treatment of disorders involving complement activity and various inflammatory and immune disorders (claimed). (I) is useful for treating neurological disorders (e.g. multiple sclerosis, stroke, Parkinson's, Alzheimer's **disease**, traumatic brain injury and allergic encephalitis), disorders of inappropriate or undesirable complement activation (e.g. xenograft rejection, corneal graft rejection), inflammatory disorders (including ulcerative colitis, Crohn's **disease**, uveitis, psoriasis, asthma, scleroderma, acute pancreatitis), post-ischemic reperfusion

conditions (e.g. myocardial infarction, **hypertension**, renal ischemia, restenosis, atherosclerosis), infectious diseases or sepsis (e.g. multiple organ failure, septic shock), autoimmune diseases (e.g. rheumatoid arthritis, systemic lupus erythematosus, hemolytic anemia, glomerulonephritis and myasthenia gravis), reproductive disorders (antibody- or complement-mediated infertility), and wound healing.

ADMINISTRATION - Administered by oral, topical, parenteral, sublingual, transdermal route or by inhalation. Dosage is 0.01-100 mg/kg, preferably 0.1-10 mg/kg.

EXAMPLE - A lipid-raft **targeted** derivative of soluble human urinary CD59 (APT632) was synthesized from soluble CD59 isolated from human urine (u-hCD59). u-hCD59 in phosphate buffered saline (PBS) was mixed with **2**-iminothiolane and the mixture was incubated at room temperature for 30 minutes. The solution was then dialyzed into PBS to remove unreacted **2**-iminothiolane, and a solution of tris-**2**-carboxyethyl phosphine was added, and the mixture was left overnight at room temperature. To this solution, 10 microlitres of APT542 (21 mM in dimethyl sulfoxide; GSSKSPSKKKKKKPGDC) was added and incubated at room temperature for **2** hours. The product APT632 was characterized by the appearance of a protein species which migrated at 21 kDa as analyzed by sodium dodecyl sulfate-polyacrylamide gel **electrophoresis** (SDS-PAGE). A reactive lysis assay demonstrated that APT632 protected guinea pig erythrocytes from complement-mediated lysis by human serum at a concentration greater than 0.5 nM. The activity of APT632 was similar to the potency of the glycosylphosphatidylinositol (GPI)-anchored form of CD59 that had been extracted from human erythrocytes.(51 pages)

L7 ANSWER 33 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-09622 BIOTECHDS
TITLE: A polynucleotide encoding a TZON7 polypeptide or a biologically active fragment for the preparation of diagnostic and pharmaceutical compositions for use in e.g., organ transplantation and in the treatment of asthma; vector-mediated recombinant protein gene transfer and expression in host cell, antibody, antisense and drug screening for **disease** and disorder diagnosis and gene therapy
AUTHOR: UTKU N
PATENT ASSIGNEE: UTKU N
PATENT INFO: WO 2002002619 10 Jan 2002
APPLICATION INFO: WO 2000-EP7610 3 Jul 2000
PRIORITY INFO: EP 2000-114234 3 Jul 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-171636 [22]
AN 2002-09622 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - A polynucleotide (P1) encoding a TZON7 polypeptide or a biologically active fragment.

DETAILED DESCRIPTION - The nucleic acid molecule is selected from the group consisting of: i) DNA sequences encoding the amino acid sequence of 39, 43, 44 and 274 and amino acids, as fully defined in the specification; ii) the DNA sequence of 135 and 825 nucleic acids, as fully defined in the specification; iii) DNA sequences encoding a fragment or derivative of the protein encoded by the DNA sequence of (i) or (ii); iv) DNA sequences the complementary strand of which hybridizes with and which is at least 70% identical to the polynucleotide as defined in (i-iii); and v) DNA sequences the nucleotide of which is degenerate to the nucleotide sequence of a DNA sequence of (i-iv). INDEPENDENT CLAIMS are also included for the following: (1) a nucleic acid molecule of at least 15 nucleotides in length hybridizing specifically with a polynucleotide (P1) or with a complementary strand, (**2**) a vector comprising the polynucleotide (P1) of the nucleic acid molecule of

claim (1); (3) a host comprising a polynucleotide (P1) or the vector of claim (2); (4) a method (M1) for the production of a TZON7 protein/polypeptide or a biologically active fragment, comprising, a) culturing the host of claim (3) under conditions allowing for the expression of the protein, or b) in vitro translation of the polynucleotide (P10, and recovering the protein produced in (a) or (b); (5) a TZON7 protein/polypeptide or a biologically active fragment encoded by the polynucleotide (P1) or produced by the method (M1); (6) an antibody specifically recognizing the protein of claim (5); (7) a normal cell that has been modified to express the protein of claim (5) or the antibody of claim (6); (8) an antisense construct capable of inhibiting the expression of the polynucleotide (P1); (9) a method (M2) of diagnosing or a susceptibility to a pathological condition in a subject related to a disorder in the immune system comprising either a) determining the presence or absence of a mutation in the polynucleotide (P1), and diagnosing the condition based on the presence or absence of the mutation, or b) determining the presence or amount of expression of the protein of claim (5) in a biological sample and diagnosing the condition based on the presence or amount or the protein; (10) a method (M3) for identifying a binding partner to a TZON7 protein comprising, contacting the protein with a compound to be screened and determining whether the compound effects an activity of the protein, (11) a method (M4) for identifying leukocyte/lymphocyte activating or co-stimulating compounds or for identifying inhibitors of leukocyte activation and stimulation comprising, a) culturing leukocytes, lymphocytes or monocytes in the presence of the protein of claim (5) the antibody of claim (6), the cell of claim (3) or (7) and optionally, in the presence of a component capable of providing a detectable signal in response to leukocyte proliferation, with a compound to be screened under conditions permitting interaction of the compound with the (poly)peptide, antibody or cell(s), and b) detecting the presence or absence of a signal generated from the interaction of the compound with the cells; (12) a method (M5) for the production of a pharmaceutical composition comprising the steps of (M3) or (M4) and formulating and optionally synthesizing the compound identified in step (b); and (13) a method (M6) for determining the status of an immune response comprising analyzing the presence of the polynucleotide (P1) or the protein of claim (5).

BIOTECHNOLOGY - Preparation: The polynucleotide was isolated using standard recombinant techniques. Preferred vector - The polynucleotide or nucleic acid molecule is operably linked to regulatory sequences allowing for the transcription and optionally, expression of the nucleic acid molecule.

ACTIVITY - Immunosuppressive; antiallergic; cytostatic; antirheumatic; antiarthritic; dermatological; antiinflammatory; neuroprotective; antidiabetic; gastrointestinal; endocrine; antithyroid; antiasthmatic; vulnerary; antibacterial.

MECHANISM OF ACTION - Immunomodulator.

USE - A pharmaceutical composition is claimed which comprises the polynucleotide (P1), the nucleic acid of claim (1) the vector of claim (2), the cell of claim (3), the protein of claim (5), the antibody of claim (6) or the antisense construct of claim (8) for use in cell or organ transplantation, for the treatment of autoimmune, allergic or infectious diseases or for the treatment of tumors or for the improvement of allograft or xenograft tolerance. A diagnostic composition is also claimed which comprises the polynucleotide (P1), the nucleic acid of claim (1) the vector of claim (2), the cell of claim (3)or (7), the protein of claim (5), the antibody of claim (6) and optionally at least one component which is labelled. The polynucleotide (P1),the nucleic acid of claim (1), the vector of claim (2), the cell of claim (3) or (7), the protein of claim (5), the antibody of claim (6) or the antisense construct of claim (8) or the compound identified by methods (M3), (M4) and (M5) are used for the preparation for the composition for diagnosing or the treatment of acute and chronic diseases, involving T cell activation and Th1 and Th2 immune response,

for the treatment of acute and chronic rejection of allo- and xeno- organ transplants and bone marrow transplantation, for the treatment of rheumatoid arthritis, lupus erythematodes, multiple sclerosis, encephalitis, vasculitis, **diabetes** mellitus, pancreatitis, gastritis, thyroiditis, for the treatment of malign disorders of T, B or NK cells, for the treatment of asthma, lepramatosis, Helicobacter pylori associated gastritis or for the treatment of skin tumors, adrenal tumors or lung tumors, wound healing, growth disorders, inflammatory and/or infectious diseases. The polynucleotide (P1) or the nucleic acid of claim (1) or the antibody of claim (6) can be used for the detection of leukocyte/lymphocyte activation. The leukocyte/lymphocyte is a B cell, T cell, NK cell and/or monocyte (all claimed).

ADMINISTRATION - Administration of the pharmaceutical compositions maybe by intravenous, intraperitoneal, subcutaneous, intramuscular, topical or intradermal routes. Dosage may be 0.001 to 1000 microg

ADVANTAGE - The complex molecular mechanisms involved in T-cell activation is not fully understood but the present invention provides information and **targets** for the therapeutic modulation of the immune response involved in autoimmune **disease** and graft rejection.

EXAMPLE - A novel cDNA fragment, TZON7 was identified that is differentially express in alloactivated T cell lines. Differential display RT-PCR (DDRT-PCR) analysis of mRNA expression was performed at time 0 and 24h after stimulation of a preconditioned human T cell line with allo-antigen. The preconditioned T cell line was prepared by isolating peripheral blood lymphocytes (PBLs) from healthy volunteers. Isolated human PBLs (responder PBLs) were stimulated with equal numbers of irradiated stimulator PBLs from another healthy individual. Cells were co-cultured and restimulated with stimulator cells three times in 10 day intervals prior to RNA isolation. Total RNA was isolated from cells at 0 and 24 h after last stimulation using the RNAzol B method and DDRT-PCR was carried out, a method which gives unbiased analysis of changes in message levels from cDNA amplified with multiple sets of primers followed by parallel 6% polyacrylamide gel **electrophoresis**. The PCR products were stored and separated in 6% polyacrylamide-urea gels, and analysed. The differentially expressed cDNA fragment was excised, eluted, reamplified, cloned into pBluescriptSK+ plasmid and sequenced. Analysis of the cDNAs showed several genes at 24 hours. One or the upregulated transcripts, TZON7, was reamplified, subcloned and 135 base pairs were sequenced. By searching GenBank, the deduced 44 amino acid sequence of TZON7 showed 18% identity to human zonadhesin molecule. (59 pages)

L7 ANSWER 34 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2003-01777 BIOTECHDS
TITLE: New human nucleic acid molecule, designated 61833, which encodes a novel pyridoxal-dependant decarboxylase, useful for treating cellular proliferative or differentiative disorders, cardiovascular disorders, and/or brain disorders; plasmid vector-mediated recombinant protein gene transfer and expression in COS cell for **disease** diagnosis and gene therapy
AUTHOR: GLUCKSMANN M A
PATENT ASSIGNEE: GLUCKSMANN M A
PATENT INFO: US 2002119913 29 Aug 2002
APPLICATION INFO: US 2001-841880 24 Apr 2001
PRIORITY INFO: US 2001-841880 24 Apr 2001; US 2000-199559 25 Apr 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-618951 [66]
AN 2003-01777 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - An isolated human nucleic acid molecule (N1), designated 61833, which encodes a novel pyridoxal-dependant decarboxylase, is new.

DETAILED DESCRIPTION - An isolated human nucleic acid molecule (N1),

designated 61833, which encodes a novel pyridoxal-dependant decarboxylase, is new. N1 is selected from: (a) a nucleic acid molecule comprising a sequence which is at least 85% identical to the 1937 (S1) or 1383 (S2) base pair (bp) sequence defined in the specification; (b) a nucleic acid molecule comprising a fragment of at least 442 nucleotides of S1 or S2; (c) a nucleic acid molecule which encodes a polypeptide comprising the 460 amino acid sequence (S3) defined in the specification; (d) a nucleic acid molecule which encodes a fragment of a polypeptide comprising sequence of S3, where the fragment comprises at least 15 contiguous amino acids of S3; or (e) a nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of S3, where the nucleic acid molecule hybridizes to a nucleic acid molecule comprising S1, S2, or its complement, under stringent conditions. INDEPENDENT CLAIMS are included for the following: (1) a host cell which contains N1; (2) a non-human mammalian host cell containing N1; (3) an isolated polypeptide (P1) selected from: (a) a polypeptide which is encoded by a nucleic acid molecule comprising a sequence which is at least 85% identical to a nucleic acid comprising the sequence of S1 or S2; (b) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of S3, where the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising S1, 2, or its complement under stringent conditions; and (c) a fragment of a polypeptide comprising the amino acid sequence of S3, where the fragment comprises at least 15 contiguous amino acids of S3; (4) an antibody which selectively binds to P1; (5) a method for producing P1 comprising culturing the host cell of (1); (6) a method for detecting the presence of P1 in a sample, comprising contacting the sample with an antibody which selectively binds to P1 and determining whether the antibody binds to the polypeptide in the sample; (7) a method (M1) for detecting the presence of N1 in a sample, comprising contacting the sample with a nucleic acid probe or primer which selectively hybridizes to the nucleic acid molecule, and determining whether the nucleic acid probe or primer binds to a nucleic acid molecule in the sample; (8) a kit comprising an antibody which selectively binds to P1 or a compound which selectively hybridizes to N1; (9) a method for identifying a compound which binds to P1 comprising contacting P1, or a cell expressing P1 with a test compound, and determining whether the polypeptide binds to the test compound; (10) a method for modulating the activity of P1 comprising contacting a polypeptide or a cell expressing P1 with a compound which binds to the polypeptide in a sufficient concentration to modulate the activity of the polypeptide; (11) a method (M2) of inhibiting the proliferation or migration, or inducing the killing, of a 61833-expressing cell, comprising contacting the cell with a compound that modulates the activity or expression of P1, in an amount which is effective to reduce or inhibit the proliferation, or induce the killing, of the cell; (12) a method of inhibiting the proliferation or migration, or inducing the killing, of a 61833-expressing cell, comprising contacting the cell with a compound that modulates the activity or expression of N1, in an amount which is effective to reduce or inhibit the proliferation, or induce the killing, of the cell; (13) a method of evaluating a sample for a proliferative disorder, comprising detecting an expression level of N1 in the sample, comparing the expression level to a reference level, where an increase in the expression level relative to the reference level is an indication of the proliferative disorder; and (14) a method of evaluating a sample for a proliferative disorder, comprising detecting an abundance of P1 in the sample, comparing the abundance to a reference level, where an increase in the abundance to the reference level is an indication of the proliferative disorder.

BIOTECHNOLOGY - Preferred Nucleic Acid: N1 comprises the sequence of S1 or S2 and it encodes a polypeptide comprising the amino acid sequence of S3. N1 further comprises a vector nucleic acid sequence. N1 further comprises a nucleic acid sequence encoding a heterologous polypeptide. Preferred Host Cell: The host cell is a mammalian host cell. Preferred

Polypeptide: P1 comprises the amino acid sequence of S3. P1 further comprises a heterologous amino acid sequence. Preferred Method: In M1, the sample comprises mRNA molecules and is contacted with a nucleic acid probe.

ACTIVITY - Cytostatic; Cardiant; Hypotensive; Antiarteriosclerotic; Antiinflammatory; Nootropic; Neuroprotective; Antiparkinsonian; Central Nervous System General; Immunsuppressive; Antidiabetic; Antipsoriatic; antiarthritic; Dermatological; Antiulcer. No biological data given.

MECHANISM OF ACTION - Modulator of 61833 expression or activity. No biological data given.

USE - A compound that modulates the activity or expression of N1 is useful for treating or preventing a disorder characterized by aberrant cellular proliferation or differentiation (claimed). The 61833 nucleic acid, polypeptide molecules and antibodies can act as novel diagnostic **targets** and therapeutic agents for controlling cellular proliferative or differentiative disorders (such as cancer, e.g., carcinoma, sarcoma, metastatic disorders or hematopoietic neoplastic disorder), cardiovascular disorders (e.g. **hypertension**, atherosclerosis, coronary artery spasm, congestive heart failure, coronary artery **disease**, valvular **disease**, arrhythmias, and cardiomyopathies), disorders of the brain (e.g. Alzheimer **disease** and Pick **disease**, Parkinsonism, idiopathic Parkinson **disease** (paralysis agitans), progressive supranuclear palsy, corticobasal degeneration, multiple system atrophy, Shy-Drager syndrome, and olivopontocerebellar atrophy, and Huntington **disease**) and/or immune disorders (e.g. autoimmune diseases (including **diabetes** mellitus, arthritis), multiple sclerosis, encephalomyelitis, myasthenia gravis, systemic lupus erythematosis, autoimmune thyroiditis, dermatitis, psoriasis, Sjogren's Syndrome, Crohn's **disease**, aphthous ulcer, iritis, and conjunctivitis).

ADMINISTRATION - The nucleic acids, polypeptides and antibodies can be administered by intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal routes.The dosage of the polypeptide ranges from 0.1 to 20 mg/kg body weight and can be administered once per week for between 1 to 10 weeks, preferably between 4, 5, or 6 weeks. For antibodies, the preferred dosage is 0.1 mg/kg of body weight (generally 10 mg/kg to 20 mg/kg). If the antibody is to act in the brain, a dosage of 50 mg/kg to 100 mg/kg is usually appropriate.

EXAMPLE - To express the 61833 gene in COS cells, the pcDNA/Amp vector by Invitrogen Corporation was used. This vector contains an SV40 (Simian virus 40) origin of replication, an ampicillin resistance gene, an E. coli replication origin, a CMV (Cauliflower mosaic virus) promoter followed by a polylinker region, and an SV40 intron and polyadenylation site. A DNA fragment encoding the entire 61833 protein and an HA (haemagluttinin) tag (Wilson et. al. (1984) Cell 37:767) or a FLAG tag fused in-frame to its 3' end of the fragment is cloned into the polylinker region of the vector, thereby placing the expression of the recombinant protein under the control of the CMV promoter. To construct the plasmid, the 61833 DNA sequence was amplified by polymerase chain reaction (PCR) using **two** primers. The 5' primer contains the restriction site of interest followed by approximately twenty nucleotides of the 61833 coding sequence starting from the initiation codon; the 3' end sequence contains complementary sequences to the other restriction site of interest, a translation stop codon, the HA tag or FLAG tag and the last 20 nucleotides of the 61833 coding sequence. The PCR amplified fragment and the pCDNA/Amp vector are digested with the appropriate restriction enzymes and the vector is dephosphorylated using the CIAP enzyme (New England Biolabs). Preferably the **two** restriction sites chosen are different so that the 61833gene is inserted in the correct orientation. The ligation mixture was transformed into E. coli cells (strains HB101, DH5-alpha, SURE, available from Stratagene Cloning Systems), the transformed culture was plated on ampicillin media plates, and resistant colonies were selected. Plasmid DNA was isolated from

transformants and examined by restriction analysis for the presence of the correct fragment. COS cells were subsequently transfected with the 61833-pcDNA/Amp plasmid DNA using the calcium phosphate or calcium chloride co-precipitation methods, DEAE-dextran-mediated transfection, lipofection, or electroporation. The expression of the 61833 polypeptide is detected by radiolabelling (35S-methionine or 35S-cysteine and immunoprecipitation (Harlow, E. and Lane, D. (1988) Antibodies. A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.) using an HA specific monoclonal antibody. Briefly, the cells are labeled for 8 hours with 35S-methionine (or 35S-cysteine). The culture media are then collected and the cells are lysed using detergents (RIPA buffer, 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% DOC, 50 mM Tris, pH 7.5). Both the cell lysate and the culture media are precipitated with an HA specific monoclonal antibody. Precipitated polypeptides are then analyzed by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel **electrophoresis**).(54 pages)

L7 ANSWER 35 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2003-01836 BIOTECHDS
TITLE: Novel polypeptide useful for diagnosis, prognosis, prevention, and treatment of immune, hyperproliferative, renal, respiratory, cardiovascular, reproductive, endocrine, gastrointestinal and neurological disorders; recombinant protein production via plasmid expression in host cell use in **disease** therapy and gene therapy
AUTHOR: ROSEN C A; RUBEN S M; BARASH S C
PATENT ASSIGNEE: ROSEN C A; RUBEN S M; BARASH S C
PATENT INFO: US 2002090673 11 Jul 2002
APPLICATION INFO: US 2001-764898 17 Jan 2001
PRIORITY INFO: US 2001-764898 17 Jan 2001; US 2000-179065 31 Jan 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-642377 [69]
AN 2003-01836 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - An isolated polypeptide (I) comprising a sequence at least 90% identical to a sequence selected from a full length protein sequence (S1) given in the specification, or the encoded sequence (ES) contained in cDNA clone (ID Nos. given in the specification, such as HPLBY29), a fragment, domain or epitope of S1 or ES, or a variant, allelic variant or species homolog of S1, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated nucleic acid molecule (II) comprising a nucleotide sequence at least 95% identical to a polynucleotide fragment having a sequence (S2) given in the specification, a polynucleotide encoding a polypeptide, polypeptide fragment, domain or epitope of S1 or the cDNA sequence contained in (C) which is hybridizable to S2, having biological activity, a polynucleotide which encodes the species homolog of S1, a polynucleotide which is the variant or allelic variant of S2, or a polynucleotide capable of hybridizing under stringent conditions to any one of the above polynucleotides, where the polynucleotide does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide sequence of only A or T residues; (**2**) a recombinant vector comprising (II); (3) making a recombinant host cell comprising (II); (4) a recombinant host cell (HC) produced by the above method; (5) an isolated antibody (III) that binds specifically to (I); (6) a recombinant host cell (IV) that expresses (I); (7) preparing (I); (8) a polynucleotide produced by the above method; (9) a gene corresponding to cDNA sequence of S2; (10) identifying an activity in a biological sample, by expressing S2 in a cell, isolating the supernatant, detecting an activity in a biological sample and identifying the protein in the supernatant having the activity; and (11) a product produced by the above method.

WIDER DISCLOSURE - Also disclosed are: (1) T-cell antigen receptors which immunospecifically bind (I); (2) polynucleotides comprising nucleotide sequence encoding (III); (3) antibodies recombinantly fused or chemically conjugated to (I); (4) compositions comprising (I) fused or conjugated to antibody domains other than the variable domains; (5) fragments of (III); (6) a pharmaceutical pack or kit comprising containers filled with pharmaceutical composition comprising (I), (II) or (III); (7) polypeptides which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation; and (8) chemically modified derivatives of (I).

BIOTECHNOLOGY - Preparation: (I) is prepared by culturing (IV) under conditions such that the polypeptide is expressed and recovering the polypeptide. Preferred Polypeptide: The full length protein comprises sequential amino acid deletions from either the C- or N-terminus. Preferred Nucleic Acid Molecule: The polynucleotide fragment in (II) comprises a nucleotide sequence encoding a protein. (II) comprises sequential nucleotide deletions from the C- or N-terminus. Preferred Host Cell: HC comprises vector sequences.

ACTIVITY - Immunostimulant; Antirheumatic; Antiarthritic; Neuroprotective; Antiallergic; Antidiabetic; Antiasthmatic; Antiinflammatory; Immunosuppressive; Anticoagulant; Thrombolytic; Antiatherosclerotic; Cytostatic; Nephrotropic; Nootropic; Antiparkinsonian; Gynecological; Virucide; Antibacterial; Antiarrhythmic; Fungicide. Test details are given, but no results are given.

MECHANISM OF ACTION - Gene therapy. No supporting data provided.

USE - (I) and (II) are useful for diagnosing a pathological condition or susceptibility to a pathological condition in a subject, where the presence or absence of a mutation in (II) is determined, or where the amount of expression of (I) is determined, and for preventing, treating or ameliorating a medical condition. (I) is also useful for identifying a binding partner to the polypeptide (all claimed). (I), (II) and (III) are useful in treating, preventing, diagnosing and/or prognosing immunodeficiencies (e.g., B cell immunodeficiencies, severe combined immunodeficiencies), autoimmune disorders (rheumatoid arthritis, multiple sclerosis, **diabetes** mellitus), allergic reactions and conditions (e.g., asthma), inflammatory conditions, graft-versus-host **disease**, blood-related disorders (thrombosis, atherosclerosis), hyperproliferative disorders (e.g., cancer), renal disorders (e.g., acute glomerulonephritis), cardiovascular disorders (e.g., arrhythmia), respiratory disorders (Goodpasture's syndrome), neurological disorders (e.g., Alzheimer's **disease**, Parkinson's **disease**), endocrine disorders (e.g., Addison's **disease**), reproductive system disorders (e.g., endometriosis), infectious diseases (e.g., viral, bacterial or fungal infections), and gastrointestinal disorders (e.g., Crohn's **disease**). (I) is useful to stimulate neuronal growth and treat, prevent, and/or diagnose neuronal damage which occurs in certain neuronal disorders or neuro-degenerative conditions, for stimulating keratinocyte growth, to prevent hair loss, to modulate mammalian characteristics such as body height, weight, hair color, and to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional components. (I) is also useful as a molecular weight **marker** on sodium dodecyl sulfate-polyacrylamide gel **electrophoresis** (SDS-PAGE) gels, and to raise antibodies. (II) is useful for chromosome identification, radiation hybrid mapping, in gene therapy, for identifying individuals from minute biological samples, as additional DNA **markers** for restriction fragment length polymorphism (RFLP), in forensic biology, as molecular weight **markers** on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response. (III) is useful for immunophenotyping cell lines and biological samples and for diagnosing and treating diseases, disorders or conditions. (III) is also

useful to assay protein levels in a biological sample.

ADMINISTRATION - (III) is administered at a dose of 0.1-100 mg/kg and (II) is administered at a dose of 0.05 mg-50 mg/kg. (I), (II) or (III) is administered by intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, or oral route.

EXAMPLE - No relevant example is given. (252 pages)

L7 ANSWER 36 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2003-01820 BIOTECHDS
TITLE: Novel respiratory system related polypeptide useful for diagnosis, prognosis, prevention, and treatment of immune, hyperproliferative, renal, respiratory, cardiovascular, reproductive or gastrointestinal disorders; human recombinant protein production and antibody useful for gene therapy, diagnosis and prognosis
AUTHOR: ROSEN C A; RUBEN S M; BARASH S C
PATENT ASSIGNEE: ROSEN C A; RUBEN S M; BARASH S C
PATENT INFO: US 2002086820 4 Jul 2002
APPLICATION INFO: US 2001-764862 17 Jan 2001
PRIORITY INFO: US 2001-764862 17 Jan 2001; US 2000-179065 31 Jan 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-635684 [68]
AN 2003-01820 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - An isolated respiratory system related polypeptide (I) comprising a sequence at least 90% identical to a full length protein sequence (S1) of 157, 337 or 112 amino acids fully defined in the specification, or the encoded sequence (ES) contained in cDNA clone ID Nos.HPMKW36 or HEOQR40, or a fragment, domain or epitope of S1 or ES, or a variant, allelic variant or species homolog of S1, is new.

DETAILED DESCRIPTION - An isolated polypeptide (I) comprises a sequence at least 90% identical to a sequence selected from: (a) a full length protein having a sequence (S1) of 157, 337 or 112 amino acids defined in the specification, or the encoded sequence (ES) contained in cDNA clone (C) ID Nos.HPMKW36 or HEOQR40; (b) a polypeptide fragment, domain or epitope of S1 or ES; and (c) a variant, allelic variant or species homolog of S1. INDEPENDENT CLAIMS are also included for the following: (1) an isolated nucleic acid molecule (II) comprising a nucleotide sequence at least 95% identical to a polynucleotide fragment having a sequence (S2) of 1102, 1666 or 1591 base pairs fully defined in the specification, a polynucleotide encoding a polypeptide, polypeptide fragment, domain or epitope of S1 or the cDNA sequence contained in (C) which is hybridizable to S2, having biological activity, a polynucleotide which encodes the species homolog of S1, a polynucleotide which is the variant or allelic variant of S2, or a polynucleotide capable of hybridizing under stringent conditions to any one of the above polynucleotides, where the polynucleotide does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide sequence of only A or T residues; (2) a recombinant vector comprising (II); (3) making a recombinant host cell comprising (II); (4) a recombinant host cell (HC) produced by the above method; (5) an isolated antibody (III) that binds specifically to (I); (6) a recombinant host cell (IV) that expresses (I); (7) preparing (I); (8) a polynucleotide produced by the above method; (9) a gene corresponding to cDNA sequence of S2; (10) identifying an activity in a biological sample, by expressing S2 in a cell, isolating the supernatant, detecting an activity in a biological sample and identifying the protein in the supernatant having the activity; and (11) a product produced by the above method.

WIDER DISCLOSURE - Also disclosed are: (1) T-cell antigen receptors which immunospecifically bind (I); (2) polynucleotides comprising nucleotide sequence encoding (III); (3) antibodies recombinantly fused or chemically conjugated to (I); (4) compositions

comprising (I) fused or conjugated to antibody domains other than the variable domains; (5) fragments of (III); (6) a pharmaceutical pack or kit comprising containers filled with pharmaceutical composition comprising (I), (II) or (III); (7) polypeptides which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation; and (8) chemically modified derivatives of (I).

BIOTECHNOLOGY - Preparation: (I) is prepared by culturing (IV) under conditions such that the polypeptide is expressed and recovering the polypeptide (claimed). Preferred Polypeptide: The secreted form or the full length protein comprises sequential amino acid deletions from either the C- or N-terminus. Preferred Nucleic Acid Molecule: The polynucleotide fragment in (II) comprises a nucleotide sequence encoding a sequential protein. (II) comprises sequential nucleotide deletions from the C- or N-terminus. Preferred Host Cell: HC comprises vector sequences.

ACTIVITY - Immunostimulant; Antirheumatic; Antiarthritic; Neuroprotective; Antiallergic; Antidiabetic; Antiasthmatic; Antiinflammatory; Immunosuppressive; Anticoagulant; Thrombolytic; Antiatherosclerotic; Cytostatic; Nephrotropic; Nootropic; Antiparkinsonian; Gynecological; Virucide; Antibacterial; Antiarrhythmic; Fungicide. Test details are given, but no results are given.

MECHANISM OF ACTION - Gene therapy.

USE - (I) and (II) are useful for diagnosing a pathological condition or susceptibility to a pathological condition in a subject and for preventing, treating or ameliorating a medical condition. (I) is also useful for identifying a binding partner to the polypeptide (claimed). (I), (II) and (III) are useful in treating, preventing, diagnosing and/or prognosing immunodeficiencies (e.g., B cell immunodeficiencies, severe combined immunodeficiencies), autoimmune disorders (rheumatoid arthritis, multiple sclerosis, **diabetes** mellitus), allergic reactions and conditions (e.g., asthma), inflammatory conditions, graft-versus-host **disease**, blood-related disorders (thrombosis, atherosclerosis), hyperproliferative disorders (e.g., cancer), renal disorders (e.g., acute glomerulonephritis), cardiovascular disorders (e.g., arrhythmia), respiratory disorders (Goodpasture's syndrome), neurological disorders (e.g., Alzheimer's **disease**, Parkinson's **disease**), endocrine disorders (e.g., Addison's **disease**), reproductive system disorders (e.g., endometriosis), infectious diseases (e.g., viral, bacterial or fungal infections), and gastrointestinal disorders (e.g., Crohn's **disease**). (I) is useful to stimulate neuronal growth and treat, prevent, and/or diagnose neuronal damage which occurs in certain neuronal disorders or neuro-degenerative conditions, for stimulating keratinocyte growth, to prevent hair loss, to modulate mammalian characteristics such as body height, weight, hair color, and to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional components. (I) is also useful as a molecular weight **marker** on sodium dodecyl sulfate-polyacrylamide gel **electrophoresis** (SDS-PAGE) gels, and to raise antibodies. (II) is useful for chromosome identification, radiation hybrid mapping, in gene therapy, for identifying individuals from minute biological samples, as additional DNA **markers** for restriction fragment length polymorphism (RFLP), in forensic biology, as molecular weight **markers** on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response. (III) is useful for immunophenotyping cell lines and biological samples and for diagnosing and treating diseases, disorders or conditions. (III) is also useful to assay protein levels in a biological sample.

ADMINISTRATION - (III) is administered at a dose of 0.1-100 mg/kg and (II) is administered at a dose of 0.05 mg-50 mg/kg. (I), (II) or (III) is administered by intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, or oral route.

EXAMPLE - Genomic clones corresponding to the novel respiratory

system related polynucleotides were isolated. A human genomic P1 library was screened by polymerase chain reaction (PCR) using primers selected for cDNA sequence corresponding to a sequence of 1102, 1666 or 1591 base pairs fully defined in the specification. Human proteins having a sequence of 157, 337 or 112 amino acids defined in the specification were isolated and characterized. (176 pages)

L7 ANSWER 37 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2003-01830 BIOTECHDS
TITLE: Novel polypeptide useful for diagnosis, prognosis, prevention, and treatment of immune, hyperproliferative, renal, respiratory, cardiovascular, reproductive, endocrine, gastrointestinal and neurological disorders; vector-mediated recombinant protein gene transfer and expression in host cell for use in gene therapy
AUTHOR: ROSEN C A; RUBEN S M; BARASH S C
PATENT ASSIGNEE: ROSEN C A; RUBEN S M; BARASH S C
PATENT INFO: US 2002086811 4 Jul 2002
APPLICATION INFO: US 2001-764861 17 Jan 2001
PRIORITY INFO: US 2001-764861 17 Jan 2001; US 2000-179065 31 Jan 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-642253 [69]
AN 2003-01830 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - An isolated polypeptide (I) comprising a sequence at least 90% identical to a full length protein sequence (S1) of 132, 402, 125, 152, 115, 140, 99, 102, 391, 184, 175, 150, 163, 166, 257, 285, 225, or 58 amino acids defined in the specification, or the encoded sequence (ES) contained in cDNA clone ID Nos. given in the specification such as HWBAO18, or a fragment, domain or epitope of S1 or ES, is new.

DETAILED DESCRIPTION - An isolated polypeptide (I) comprises a sequence at least 90% identical to a sequence selected from: (a) a full length protein having a sequence (S1) of 132, 402, 125, 152, 115, 140, 99, 102, 391, 184, 175, 150, 163, 166, 257, 285, 225, or 58 amino acids defined in the specification, or the encoded sequence (ES) contained in cDNA clone (C) ID Nos. given in the specification such as HWBAO18, HE2OI42, HMSPF61, HPMBZ21, HDPMT22, HOHBY04, HBGMR22, HLTGA03, HLTCT21, HFXBN61, HSWBV54, HE9RA75, HYAA079, HWMEC68, HWAAM48, HLWAK52 or HTEGX10; (b) a polypeptide fragment, domain or epitope of S1 or ES; and (c) a variant, allelic variant or species homolog of S1. INDEPENDENT CLAIMS are also included for the following: (1) an isolated nucleic acid molecule (II) comprising a nucleotide sequence at least 95% identical to a polynucleotide fragment having a sequence (S2) of 397, 1623, 658, 564, 468, 739, 672, 904, 1319, 574, 695, 665, 678, 781, 1012, 915, 1690, or 897 base pairs fully defined in the specification, a polynucleotide encoding a polypeptide, polypeptide fragment, domain or epitope of S1 or the cDNA sequence contained in (C) which is hybridizable to S2, having biological activity, a polynucleotide which encodes the species homolog of S1, a polynucleotide which is the variant or allelic variant of S2, or a polynucleotide capable of hybridizing under stringent conditions to any one of the above polynucleotides, where the polynucleotide does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide sequence of only A or T residues; (2) a recombinant vector comprising (II); (3) making a recombinant host cell comprising (II); (4) a recombinant host cell (HC) produced by the above method; (5) an isolated antibody (III) that binds specifically to (I); (6) a recombinant host cell (IV) that expresses (I); (7) preparing (I); (8) a polypeptide produced by the above method; (9) a gene corresponding to cDNA sequence of S2; (10) identifying an activity in a biological sample, by expressing S2 in a cell, isolating the supernatant, detecting an activity in a biological sample and identifying the protein in the supernatant having the activity; and (11) a product produced by the above method.

WIDER DISCLOSURE - Also disclosed are: (1) T-cell antigen receptors which immunospecifically bind (I); (2) polynucleotides comprising nucleotide sequence encoding (III); (3) antibodies recombinantly fused or chemically conjugated to (I); (4) compositions comprising (I) fused or conjugated to antibody domains other than the variable domains; (5) fragments of (III); (6) a pharmaceutical pack or kit comprising containers filled with pharmaceutical composition comprising (I), (II) or (III); (7) polypeptides which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation; and (8) chemically modified derivatives of (I).

BIOTECHNOLOGY - Preparation: (I) is prepared by culturing (IV) under conditions such that the polypeptide is expressed and recovering the polypeptide (claimed). Preferred Polypeptide: The secreted form or the full length protein comprises sequential amino acid deletions from either the C- or N-terminus. Preferred Nucleic Acid Molecule: The polynucleotide fragment in (II) comprises a nucleotide sequence encoding a sequential protein. (II) comprises sequential nucleotide deletions from the C- or N-terminus. Preferred Host Cell: HC comprises vector sequences.

ACTIVITY - Immunostimulant; Antirheumatic; Antiarthritic; Neuroprotective; Antiallergic; Antidiabetic; Antiasthmatic; Antiinflammatory; Immunosuppressive; Anticoagulant; Thrombolytic; Antiatherosclerotic; Cytostatic; Nephrotropic; Nootropic; Antiparkinsonian; Gynecological; Virucide; Antibacterial; Antiarrhythmic; Fungicide. Test details are given, but no results are given.

MECHANISM OF ACTION - Gene therapy.

USE - (I) and (II) are useful for diagnosing a pathological condition or susceptibility to a pathological condition in a subject and for preventing, treating or ameliorating a medical condition. (I) is also useful for identifying a binding partner to the polypeptide (claimed). (I), (II) and (III) are useful in treating, preventing, diagnosing and/or prognosing immunodeficiencies (e.g., B cell immunodeficiencies, severe combined immunodeficiencies), autoimmune disorders (rheumatoid arthritis, multiple sclerosis, **diabetes** mellitus), allergic reactions and conditions (e.g., asthma), inflammatory conditions, graft-versus-host **disease**, blood-related disorders (thrombosis, atherosclerosis), hyperproliferative disorders (e.g., cancer), renal disorders (e.g., acute glomerulonephritis), cardiovascular disorders (e.g., arrhythmia), respiratory disorders (Goodpasture's syndrome), neurological disorders (e.g., Alzheimer's **disease**, Parkinson's **disease**), endocrine disorders (e.g., Addison's **disease**), reproductive system disorders (e.g., endometriosis), infectious diseases (e.g., viral, bacterial or fungal infections), and gastrointestinal disorders (e.g., Crohn's **disease**). (I) is useful to stimulate neuronal growth and treat, prevent, and/or diagnose neuronal damage which occurs in certain neuronal disorders or neuro-degenerative conditions, for stimulating keratinocyte growth, to prevent hair loss, to modulate mammalian characteristics such as body height, weight, hair color, and to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional components. (I) is also useful as a molecular weight **marker** on sodium dodecyl sulfate-polyacrylamide gel **electrophoresis** (SDS-PAGE) gels, and to raise antibodies. (II) is useful for chromosome identification, radiation hybrid mapping, in gene therapy, for identifying individuals from minute biological samples, as additional DNA **markers** for restriction fragment length polymorphism (RFLP), in forensic biology, as molecular weight **markers** on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response. (III) is useful for immunophenotyping cell lines and biological samples and for diagnosing and treating diseases, disorders or conditions. (III) is also useful to assay protein levels in a biological sample.

ADMINISTRATION - (III) is administered at a dose of 0.1-100 mg/kg

and (II) is administered at a dose of 0.05 mg-50 mg/kg. (I), (II) or (III) is administered by intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, or oral route.

EXAMPLE - Genomic clones corresponding to the novel polynucleotides were isolated. A human genomic P1 library was screened by polymerase chain reaction (PCR) using primers selected for cDNA sequence corresponding to a sequence of 397, 1623, 658, 564, 468, 739, 672, 904, 1319, 574, 695, 665, 678, 781, 1012, 915, 1690, or 897 base pairs fully defined in the specification. Human proteins having a sequence of 132, 402, 125, 152, 115, 140, 99, 102, 391, 184, 175, 150, 163, 166, 257, 285, 225, or 58 amino acids defined in the specification were isolated and characterized. (258 pages)

L7 ANSWER 38 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2003-01826 BIOTECHDS
TITLE: Novel polypeptide useful for diagnosis, prognosis, prevention, and treatment of immune, hyperproliferative, renal, respiratory, cardiovascular, reproductive, endocrine, gastrointestinal and neurological disorders; vector-mediated recombinant protein gene transfer and expression in host cell for use in **disease** diagnosis, prognosis, prevention, therapy and gene therapy
AUTHOR: ROSEN C A; RUBEN S M; BARASH S C
PATENT ASSIGNEE: ROSEN C A; RUBEN S M; BARASH S C
PATENT INFO: US 2002086353 4 Jul 2002
APPLICATION INFO: US 2001-764856 17 Jan 2001
PRIORITY INFO: US 2001-764856 17 Jan 2001; US 2000-179065 31 Jan 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-642242 [69]
AN 2003-01826 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - An isolated polypeptide (I) comprising a sequence at least 90% identical to a full length protein sequence (S1) of 69, 184, 114, 88, 130, 163, 89, 123, 142, 120, 105, 170, 200, 294, 227, 152, 170, 557, 183, 272, 48, 266, 167, 253, 264, 192, 120, 446, 170, 102, 63 or 659 amino acids fully defined in the specification, or the encoded sequence (ES) contained in cDNA clone ID Nos. given in specification, is new.

DETAILED DESCRIPTION - An isolated polypeptide (I) comprises a sequence at least 90% identical to a sequence selected from: (a) a full length protein having a sequence (S1) of 69, 184, 114, 88, 130, 163, 89, 123, 142, 120, 105, 170, 200, 294, 227, 152, 170, 557, 183, 272, 48, 266, 167, 253, 264, 192, 120, 446, 170, 102, 63 or 659 amino acids defined in the specification, or the encoded sequence (ES) contained in cDNA clone (C) ID Nos. given in the specification selected from 32 clones such as HSLE27, HMSCM39 or HSID67; (b) a polypeptide fragment, domain or epitope of S1 or ES; and (c) a variant, allelic variant or species homolog of S1. INDEPENDENT CLAIMS are also included for the following: (1) an isolated nucleic acid molecule (II) comprising a nucleotide sequence at least 95% identical to a polynucleotide fragment having a sequence (S2) of 530, 712, 352, 444, 561, 568, 539, 503, 682, 511, 411, 512, 747, 1217, 818, 810, 680, 2119, 899, 1482, 173, 1089, 810, 761, 1330, 577, 362, 1655, 764, 432, 372 or 3456 base pairs fully defined in the specification, a polynucleotide encoding a polypeptide, polypeptide fragment, domain or epitope of S1 or the cDNA sequence contained in (C) which is hybridizable to S2, having biological activity, a polynucleotide which encodes the species homolog of S1, a polynucleotide which is the variant or allelic variant of S2, or a polynucleotide capable of hybridizing under stringent conditions to any one of the above polynucleotides, where the polynucleotide does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide sequence of only A or T residues; (**2**) a recombinant vector comprising (II); (3) making a recombinant host cell comprising (II); (4) a recombinant host cell (HC) produced by the above method; (5) an isolated antibody (III) that binds specifically

to (I); (6) a recombinant host cell (IV) that expresses (I); (7) preparing (I); (8) a polypeptide produced by the above method; (9) a gene corresponding to cDNA sequence of S2; (10) identifying an activity in a biological sample, by expressing S2 in a cell, isolating the supernatant, detecting an activity in a biological sample and identifying the protein in the supernatant having the activity; and (11) a product produced by the above method.

WIDER DISCLOSURE - Also disclosed are: (1) T-cell antigen receptors which immunospecifically bind (I); (2) polynucleotides comprising nucleotide sequence encoding (III); (3) antibodies recombinantly fused or chemically conjugated to (I); (4) compositions comprising (I) fused or conjugated to antibody domains other than the variable domains; (5) fragments of (III); (6) a pharmaceutical pack or kit comprising containers filled with pharmaceutical composition comprising (I), (II) or (III); (7) polypeptides which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation; and (8) chemically modified derivatives of (I).

BIOTECHNOLOGY - Preparation: (I) is prepared by culturing (IV) under conditions such that the polypeptide is expressed and recovering the polypeptide (claimed). Preferred Polypeptide: The full length protein comprises sequential amino acid deletions from either the C- or N-terminus. Preferred Nucleic Acid Molecule: The polynucleotide fragment in (II) comprises a nucleotide sequence encoding a sequential protein. (II) comprises sequential nucleotide deletions from the C- or N-terminus. Preferred Host Cell: HC comprises vector sequences.

ACTIVITY - Immunostimulant; Antirheumatic; Antiarthritic; Neuroprotective; Antiallergic; Antidiabetic; Antiasthmatic; Antiinflammatory; Immunosuppressive; Anticoagulant; Thrombolytic; Antiatherosclerotic; Cytostatic; Nephrotropic; Nootropic; Antiparkinsonian; Gynecological; Virucide; Antibacterial; Antiarrhythmic; Fungicide. Test details are given, but no results are given.

MECHANISM OF ACTION - Gene therapy.

USE - (I) and (II) are useful for diagnosing a pathological condition or susceptibility to a pathological condition in a subject and for preventing, treating or ameliorating a medical condition. (I) is also useful for identifying a binding partner to the polypeptide (claimed). (I), (II) and (III) are useful in treating, preventing, diagnosing and/or prognosing immunodeficiencies (e.g., B cell immunodeficiencies, severe combined immunodeficiencies), autoimmune disorders (rheumatoid arthritis, multiple sclerosis, **diabetes** mellitus), allergic reactions and conditions (e.g., asthma), inflammatory conditions, graft-versus-host **disease**, blood-related disorders (thrombosis, atherosclerosis), hyperproliferative disorders (e.g., cancer), renal disorders (e.g., acute glomerulonephritis), cardiovascular disorders (e.g., arrhythmia), respiratory disorders (Goodpasture's syndrome), neurological disorders (e.g., Alzheimer's **disease**, Parkinson's **disease**), endocrine disorders (e.g., Addison's **disease**), reproductive system disorders (e.g., endometriosis), infectious diseases (e.g., viral, bacterial or fungal infections), and gastrointestinal disorders (e.g., Crohn's **disease**). (I) is useful to stimulate neuronal growth and treat, prevent, and/or diagnose neuronal damage which occurs in certain neuronal disorders or neuro-degenerative conditions, for stimulating keratinocyte growth, to prevent hair loss, to modulate mammalian characteristics such as body height, weight, hair color, and to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional components. (I) is also useful as a molecular weight **marker** on sodium dodecyl sulfate-polyacrylamide gel **electrophoresis** (SDS-PAGE) gels, and to raise antibodies. (II) is useful for chromosome identification, radiation hybrid mapping, in gene therapy, for identifying individuals from minute biological samples, as additional DNA **markers** for restriction fragment length polymorphism (RFLP), in forensic biology, as molecular weight **markers** on Southern gels,

as diagnostic probes for the presence of a specific mRNA in a particular cell type, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response. (III) is useful for immunophenotyping cell lines and biological samples and for diagnosing and treating diseases, disorders or conditions. (III) is also useful to assay protein levels in a biological sample.

ADMINISTRATION - (III) is administered at a dose of 0.1-100 mg/kg and (II) is administered at a dose of 0.05 mg-50 mg/kg. (I), (II) or (III) is administered by intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, or oral route.

EXAMPLE - Genomic clones corresponding to the novel polynucleotides were isolated. A human genomic P1 library was screened by polymerase chain reaction (PCR) using primers selected for cDNA sequence corresponding to a sequence of 530, 712, 352, 444, 561, 568, 539, 503, 682, 511, 411, 512, 747, 1217, 818, 810, 680, 2119, 899, 1482, 173, 1089, 810, 761, 1330, 577, 362, 1655, 764, 432, 372 or 3456 base pairs fully defined in the specification. Human proteins having a sequence of 69, 184, 114, 88, 130, 163, 89, 123, 142, 120, 105, 170, 200, 294, 227, 152, 170, 557, 183, 272, 48, 266, 167, 253, 264, 192, 120, 446, 170, 102, 63 or 659 amino acids defined in the specification were isolated and characterized. (296 pages)

L7 ANSWER 39 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2003-04155 BIOTECHDS
TITLE: New colorectal cancer polypeptide for diagnosing, prognosing, preventing, and treating immune, hyperproliferative, liver, kidney, reproductive disorders and for identifying modulators of therapeutic use;
recombinant protein production for use in therapy, gene therapy, recombinant vaccine and nucleic acid vaccine and for drug screening
AUTHOR: ROSEN C A; RUBEN S M; BARASH S C
PATENT ASSIGNEE: ROSEN C A; RUBEN S M; BARASH S C
PATENT INFO: US 2002119919 29 Aug 2002
APPLICATION INFO: US 2001-764855 17 Jan 2001
PRIORITY INFO: US 2001-764855 17 Jan 2001; US 2000-179065 31 Jan 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-731367 [79]
AN 2003-04155 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - An isolated polypeptide (I) comprising an amino acid sequence 90 % identical to 74 sequences of e.g. HCLHD88, HCQCR67, HCRMC26, HCRMJ47 and HCRMP18, their fragments, polypeptide domains, epitopes, variants, allelic variants, full length proteins, species homologs or the encoded sequence of a defined amino acid sequence (S1) given in specification, is new.

DETAILED DESCRIPTION - A new isolated polypeptide (I) comprises a sequence 90 % identical to a sequence (S1) chosen from 74 sequences containing defined amino acids given in the specification (their fragments, polypeptide domains, epitopes, variants, allelic variants, full length proteins, species homologs or encoded sequences). INDEPENDENT CLAIMS are also included for the following: (1) an isolated nucleic acid (NA) molecule (II) comprising: (a) a nucleotide sequence 95 % identical to a polynucleotide fragment having a sequence (S2) chosen from 74 sequences of defined base pairs (bp), given in the specification; (b) a polynucleotide encoding (I); (c) a polynucleotide which is the variant or allelic variant of (II); or (d) a polynucleotide capable of hybridizing under stringent conditions to any one of (a) - (c), which does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide sequence of only a or t residues; (**2**) a recombinant vector comprising (II); (3) making a recombinant host cell comprising (II); (4) a recombinant host cell produced by (3); (5) an isolated antibody (III) that binds specifically to (I); (6) a recombinant host

cell (IV) that expresses (I); (7) preparing (I); (8) the polypeptide produced by (7); (9) the gene corresponding to the cDNA sequence of (S2); (10) identifying a binding partner to (I) comprising: (a) contacting (I) with a binding partner; and (b) determining whether the binding partner effects an activity of (I); (11) identifying an activity in a biological sample, comprising: (a) expressing (S2) in a cell; (b) isolating the supernatant; (c) detecting an activity in a biological assay; and (d) identifying the protein in the supernatant having the activity; and (12) the product produced by (10).

WIDER DISCLOSURE - Also disclosed are: (1) polynucleotides comprising nucleotide sequence encoding (III); (2) antibodies recombinantly fused or chemically conjugated to (I); (3) compositions comprising (I) fused or conjugated to antibody domains other than the variable domains; (4) fragments of (III); (5) a kit comprising containers filled with pharmaceutical composition comprising (I), (II) or (III); (6) polypeptides which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation; and (7) chemically modified derivatives of (I).

BIOTECHNOLOGY - Preparation: (I) is prepared by culturing (IV) under conditions so that the polypeptide is expressed and recovering the polypeptide (claimed). Preferred Polypeptide: The full length protein comprises sequential amino acid deletions from either the C or N-terminus. Preferred Nucleic Acid: The polynucleotide fragment in (II) comprises a nucleotide sequence encoding a protein. (II) comprises sequential nucleotide deletions from the C or N-terminus.

ACTIVITY - Immunostimulant; Immunosuppressive; Dermatological; Antirheumatic; Antiarthritic; Neuroprotective; Antithyroid; Antianemic; Antidiabetic; Nephrotropic; Antiinflammatory; Antibacterial; Vasotropic; Vulnerary; Antiasthmatic; Antiallergic; Cytostatic; Cerebroprotective; Antiparkinsonian; Nootropic; Cardiant; Antiatherosclerotic; Anti-HIV; Hepatotropic; Antigout; Tranquilizer; Virucide; Fungicide; Antiparasitic. Test details are described but no results are given.

MECHANISM OF ACTION - Gene therapy; Antibody therapy; B cell responsiveness stimulator; T cells activator; Cytokine stimulator; Complement mediated cell lysis modulator; Angiogenesis stimulator; Neuronal growth stimulator; Vaccine.

USE - (I) and nucleic acid (II) encoding (I) are used to diagnose a pathological condition or susceptibility to a pathological condition in a subject and to prevent, treat or ameliorate a medical condition. (I) is used to identify a binding partner to the polypeptide (claimed). (II) is used for chromosome identification, radiation hybrid mapping, in gene therapy, for identifying individuals from minute biological samples, as additional DNA **markers** for restriction fragment length polymorphism (RFLP), in forensic biology, molecular weight **markers** on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response. An antibody (III) to (I) is used to purify, detect and **target** the polypeptide including both in vitro and in vivo diagnostic and therapeutic methods, and also in an immunoassay for quantitatively and qualitatively measuring levels of polypeptide in the biological sample. (III) is used for immunophenotyping cell lines and biological samples and for diagnosing and treating diseases, disorders or conditions. (I), (II) and (III) are used in treating, preventing, diagnosing and/or prognosing immunodeficiencies, e.g., X-linked agammaglobulinemia, B cell immunodeficiencies, severe combined immunodeficiencies, autoimmune disorders e.g., systemic erythematosus, rheumatoid arthritis, multiple sclerosis, autoimmune thyroiditis, autoimmune hemolytic anemia, Goodpasture's syndrome, Grave's **disease**, **diabetes** mellitus, dermatitis, hematopoietic disorders, inflammatory conditions including septic shock, sepsis, reperfusion injury, inflammatory bowel **disease**, Crohn's **disease**, respiratory disorders (e.g., asthma and allergy), gastrointestinal disorders (e.g., inflammatory bowel **disease**)

cancers (e.g., gastric, ovarian, lung, bladder, liver and breast), central nervous system (CNS) disorders e.g., multiple sclerosis, ischemic brain injury and/or stroke, traumatic brain injury, neurodegenerative disorders e.g., Parkinson's **disease** and Alzheimer's **disease**, acquired immunodeficiency syndrome (AIDS)-related dementia, and prion **disease**, cardiovascular disorders e.g., atherosclerosis, myocarditis, cardiovascular **disease**, and cardiopulmonary bypass complications, as well as many additional diseases, conditions, and disorders that are characterized by inflammation e.g., hepatitis, rheumatoid arthritis, gout, trauma, pancreatitis, sarcoidosis, dermatitis, and allogenic transplant rejection. (I), (II) and (III) are used in treating a blood-related disorder (thrombosis, or atherosclerosis), hyperproliferative disorders, renal disorders. e.g. acute glomerulonephritis, endocrine disorders e.g., Addison **disease**, hyperthyroidism, hyperpituitarism, reproductive system disorders e.g. endometriosis, infectious diseases, and pancreatic disorders. They are also used as a vaccine adjuvant that enhances immune responsiveness to an antigen, as à adjuvant to enhance tumor-specific immune responses, anti-viral, anti-bacterial, anti-fungal, anti-parasitic immune responses. They are used as stimulators of B cell responsiveness to pathogens, as an activator of T cells, as an agent to boost immunoresponsiveness among aged populations and/or neonates, as a stimulator of cytokines, to enhance or inhibit complement mediated cell lysis, for stimulating wound and tissue repair, angiogenesis, and the repair of vascular or lymphatic diseases or disorders. (I) stimulates neuronal growth and treats, prevents, and/or diagnoses neuronal damage which occurs in certain neuronal disorders or neuro-degenerative conditions, stimulates keratinocyte growth, prevents hair loss, modulates mammalian characteristics such as body height, weight, hair color, and increases or decreases storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional components. (I) is used as a molecular weight **marker** on sodium dodecyl sulfate-polyacrylamide gel **electrophoresis** (SDS-PAGE) gels, and raises antibodies.

ADMINISTRATION - Administered by intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, or oral route. An antibody (III) to (I) is administered at a dose of 0.1 - 100 mg/kg/body weight, preferably 0.1 - 20 mg/kg/body weight and most preferably 1 - 10 mg/kg/body weight.

EXAMPLE - Genomic clones corresponding to human secreted polynucleotides were isolated. A human genomic P1 library was screened by a polymerase chain reaction (PCR) using primers selected for a cDNA sequence corresponding to one of 74 sequences of e.g. HCLHD88, HCQCR67, HCRMC26, HCRMJ47 and HCRMP18 with defined base pairs, given in the specification. (183 pages)

L7 ANSWER 40 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2003-03133 BIOTECHDS
TITLE: Novel polypeptide useful for diagnosis, prognosis, prevention, and treatment of immune, hyperproliferative, renal, respiratory, cardiovascular, reproductive, endocrine, gastrointestinal and neurological disorders; vector-mediated gene transfer and expression in host cell for recombinant protein production, drug screening and gene therapy
AUTHOR: ROSEN C A; RUBEN S M; BARASH S C
PATENT ASSIGNEE: ROSEN C A; RUBEN S M; BARASH S C
PATENT INFO: US 2002090672 11 Jul 2002
APPLICATION INFO: US 2001-764853 17 Jan 2001
PRIORITY INFO: US 2001-764853 17 Jan 2001; US 2000-179065 31 Jan 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-681727 [73]
AN 2003-03133 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - An isolated polypeptide (I) comprising at least 90 % identity to a full length protein sequence (S1), not given in the specification, or the encoded sequence (ES) contained in cDNA clones, given in the specification such as HNHAM52, a fragment, domain or epitope of S1 or ES, or a variant, allelic variant or species homolog of S1, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated nucleic acid molecule (II) comprising at least 95 % identity to a polynucleotide fragment having a sequence (S2), not given in the specification, a polynucleotide encoding a polypeptide, polypeptide fragment, domain or epitope of S1 or the cDNA sequence contained in (C) which is hybridizable to S2, having biological activity, a polynucleotide which encodes the species homolog of S1, a polynucleotide which is the variant or allelic variant of S2, or a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides, where the polynucleotide does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide sequence of only A or T residues; (2) a recombinant vector comprising (II); (3) making a recombinant host cell comprising (II); (4) a recombinant host cell (HC) produced by the method of (3); (5) an isolated antibody (III) that binds specifically to (I); (6) a recombinant host cell (IV) that expresses (I); (7) preparing (I); (8) a polynucleotide produced by the method of (7); (9) a gene corresponding to cDNA sequence of S2; (10) identifying an activity in a biological sample, by expressing S2 in a cell, isolating the supernatant, detecting an activity in a biological sample and identifying the protein in the supernatant having the activity; and (11) a product produced by the method of (10).

WIDER DISCLOSURE - (1) T-cell antigen receptors which immunospecifically bind (I); (2) polynucleotides comprising nucleotide sequence encoding (III); (3) antibodies recombinantly fused or chemically conjugated to (I); (4) compositions comprising (I) fused or conjugated to antibody domains other than the variable domains; (5) fragments of (III); (6) a pharmaceutical pack or kit comprising containers filled with pharmaceutical composition comprising (I), (II) or (III); (7) polypeptides which are differentially modified during or after translation, e.g. by glycosylation, acetylation, phosphorylation; and (8) chemically modified derivatives of (I).

BIOTECHNOLOGY - Preparation: (I) is prepared by culturing (IV) under conditions so that the polypeptide is expressed and recovering the polypeptide (claimed). Preferred Polypeptide: The full length protein comprises sequential amino acid deletions from either the C- or N-terminus. Preferred Nucleic Acid: The polynucleotide fragment in (II) comprises a nucleotide sequence encoding a protein. (II) comprises sequential nucleotide deletions from the C- or N-terminus. Preferred Host Cell: HC comprises vector sequences.

ACTIVITY - Immunostimulant; Antirheumatic; Antiarthritic; Neuroprotective; Antiallergic; Antidiabetic; Antiasthmatic; Antiinflammatory; Immunosuppressive; Anticoagulant; Thrombolytic; Antiatherosclerotic; Cytostatic; Nephrotropic; Nootropic; Antiparkinsonian; Gynecological; Virucide; Antibacterial; Antiarrhythmic; Fungicide. No biological data is given.

MECHANISM OF ACTION - Gene therapy.

USE - (I) and (II) are useful for diagnosing a pathological condition or susceptibility to a pathological condition in a subject and for preventing, treating or ameliorating a medical condition. (I) is also useful for identifying a binding partner to the polypeptide. (All claimed). (I), (II) and (III) are useful in treating, preventing, diagnosing and/or prognosing immunodeficiencies (e.g. B cell immunodeficiencies, severe combined immunodeficiencies), autoimmune disorders (rheumatoid arthritis, multiple sclerosis, **diabetes** mellitus), allergic reactions and conditions (e.g. asthma), inflammatory conditions, graft-versus-host **disease**, blood-related disorders (thrombosis, atherosclerosis), hyperproliferative disorders (e.g.

cancer), renal disorders (e.g. acute glomerulonephritis), cardiovascular disorders (e.g. arrhythmia), respiratory disorders (Goodpasture's syndrome), neurological disorders (e.g. Alzheimer's **disease**, Parkinson's **disease**), endocrine disorders (e.g. Addison's **disease**), reproductive system disorders (e.g. endometriosis), infectious diseases (e.g. viral, bacterial or fungal infections), and gastrointestinal disorders (e.g. Crohn's **disease**). (I) is useful to stimulate neuronal growth and treat, prevent, and/or diagnose neuronal damage which occurs in certain neuronal disorders or neuro-degenerative conditions, for stimulating keratinocyte growth, to prevent hair loss, to modulate mammalian characteristics such as body height, weight, hair color, and to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional components. (I) is also useful as a molecular weight **marker** on sodium dodecyl sulfate-polyacrylamide gel **electrophoresis** (SDS-PAGE) gels, and to raise antibodies. (II) is useful for chromosome identification, radiation hybrid mapping, in gene therapy, for identifying individuals from minute biological samples, as additional DNA **markers** for restriction fragment length polymorphism (RFLP), in forensic biology, as molecular weight **markers** on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response. (III) is useful for immunophenotyping cell lines and biological samples and for diagnosing and treating diseases, disorders or conditions. (III) is also useful to assay protein levels in a biological sample.

ADMINISTRATION - (III) is administered at a dose of 0.1-100 mg/kg and (II) is administered at a dose of 0.05-50 mg/kg. (I), (II) or (III) is administered by intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, or oral route.

EXAMPLE - No relevant example is given. (369 pages)

L7 ANSWER 41 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2003-03735 BIOTECHDS
TITLE: Novel DNA-binding protein useful for diagnosis, prognosis, prevention and treatment of immune, hyperproliferative, respiratory, cardiovascular, reproductive, endocrine, gastrointestinal and neurological disorders; vector plasmid pQE-9-mediated recombinant protein gene transfer and expression in host cell for use in **disease** diagnosis, prognosis, prevention, therapy, gene therapy, recombinant vaccine and nucleic acid vaccine preparation
AUTHOR: ROSEN C A; RUBEN S M; BARASH S C
PATENT ASSIGNEE: ROSEN C A; RUBEN S M; BARASH S C
PATENT INFO: US 2002102638 1 Aug 2002
APPLICATION INFO: US 2001-764846 17 Jan 2001
PRIORITY INFO: US 2001-764846 17 Jan 2001; US 2000-179065 31 Jan 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-690611 [74]
AN 2003-03735 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - An isolated DNA-binding protein (I) comprising a sequence having at least 90 % identity to a polypeptide fragment, domain, epitope or full length protein comprising a sequence (S1) selected from the sequences, not given in the specification, encoded by a sequence (S2) contained in cDNA clone (C1) selected from the clones given in the specification, or variant, allelic variant or species homolog of (I), is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated nucleic acid molecule (II) comprising at least 95 % identity to a polynucleotide fragment comprising S2 or a sequence

(S3), not given in the specification, which hybridizes to S1, a polynucleotide encoding (I), or a polynucleotide capable of hybridizing under stringent conditions to the polynucleotides, where the polynucleotide does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide sequence of only A or T residues; (2) a recombinant vector comprising (II); (3) making a recombinant host cell comprising (II); (4) a recombinant host cell (III) produced by the method of (3), and expressing (I); (5) an isolated antibody (Ab), that binds specifically to (I); (6) preparing (I); (7) a polypeptide produced by the method of (6); (8) a gene corresponding to cDNA sequence of S1; (9) identifying an activity in a biological assay, by expressing S1 in a cell, isolating the supernatant, detecting an activity in a biological assay and identifying the protein in the supernatant having the activity; and (10) a product produced by the method of (9).

WIDER DISCLOSURE - (1) chemically modified derivatives of (I); (2) monomers and multimers of (I); (3) generating monoclonal antibodies specific to (I); (4) monoclonal antibodies produced by the method of (3); (5) polynucleotides comprising nucleotide sequence encoding Ab; (6) antibodies recombinantly fused or chemically conjugated to (I); (7) kits for use in diagnostic and screening methods, as well as for analyzing samples for the presence of proliferative and/or cancerous polynucleotides derived from the test subject; (8) antisense nucleic acids of (II); (9) T-cell antigen receptors which immunospecifically bind (I); (10) a pharmaceutical pack or kit comprising containers filled with pharmaceutical composition comprising (I), (II) or (III); and (11) screening methods for identifying polypeptides and non-polypeptides that bind (I).

BIOTECHNOLOGY - Preparation: (I) is prepared by culturing (III) under conditions such that the polypeptide is expressed and recovering the polypeptide (claimed). Preferred Sequence: (II) comprises sequential nucleotide deletions from the C- or N-terminus. (III) comprises vector sequences. (I) sequential amino acid deletions from the C- or N-terminus.

ACTIVITY - Immunostimulant; Antirheumatic; Antiarthritic; Neuroprotective; Antiallergic; Antidiabetic; Antiasthmatic; Antiinflammatory; Immunosuppressive; Anticoagulant; Thrombolytic; Antiatherosclerotic; Cytostatic; Nephrotropic; Nootropic; Antiparkinsonian; Gynecological; Virucide; Antibacterial; Antiarrhythmic; Fungicide. No biological data is given.

MECHANISM OF ACTION - Gene therapy; Vaccine.

USE - (I) and (II) are useful for diagnosing a pathological condition or susceptibility to a pathological condition in a subject, and for preventing, treating or ameliorating a medical condition. (I) is also useful for identifying a binding partner to the polypeptide. (All claimed). (I), (II) and (III) are useful in treating, preventing, diagnosing and/or prognosing immunodeficiencies (e.g. B cell immunodeficiencies, severe combined immunodeficiencies), autoimmune disorders (rheumatoid arthritis, multiple sclerosis, **diabetes** mellitus), allergic reactions and conditions (e.g. asthma), inflammatory conditions, graft-versus-host **disease**, blood-related disorders (thrombosis, atherosclerosis), hyperproliferative disorders (e.g. cancer), renal disorders (e.g. acute glomerulonephritis), cardiovascular disorders (e.g. arrhythmia), respiratory disorders (Goodpasture's syndrome), neurological disorders (e.g. Alzheimer's **disease**, Parkinson's **disease**), endocrine disorders (e.g. Addison's **disease**), reproductive system disorders (e.g. endometriosis), infectious diseases (e.g. viral, bacterial or fungal infections), and gastrointestinal disorders (e.g. Crohn's **disease**). (I) is useful to stimulate neuronal growth and treat, prevent, and/or diagnose neuronal damage which occurs in certain neuronal disorders or neuro-degenerative conditions, for stimulating keratinocyte growth, to prevent hair loss, to modulate mammalian characteristics such as body height, weight, hair color, and to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional components. (I) is also useful

as a molecular weight **marker** on sodium dodecyl sulfate-polyacrylamide gel **electrophoresis** (SDS-PAGE) gels, and to raise antibodies. (II) is useful for chromosome identification, radiation hybrid mapping, in gene therapy, for identifying individuals from minute biological samples, as additional DNA **markers** for restriction fragment length polymorphism (RFLP), in forensic biology, as molecular weight **markers** on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response. (III) is useful for immunophenotyping cell lines and biological samples and for diagnosing and treating diseases, disorders or conditions. (III) is also useful to assay protein levels in a biological sample.

ADMINISTRATION - 0.1-100, preferably 1-10 mg/kg Ab, or 0.05-50, preferably 0.05-5.0 mg/kg (II) is administered through intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural or oral route. (I) is also administered through the above said routes. No dosage is given.

EXAMPLE - Polynucleotide encoding the DNA-binding protein was amplified using polymerase chain reaction (PCR) oligonucleotide primers corresponding to the 5' and 3' ends of the DNA sequence, to synthesize insertion fragments. The primers used to amplify the cDNA insert contained BamHI and XbaI restriction sites at their 5' end in order to clone the amplified product into the expression vector pQE-9. The vector encodes antibiotic resistance (Ampr), a bacterial origin of replication (ori), isopropyl-B-D-thiogalactopyranoside (IPTG)-regulatable promoter/operator (P/O), ribosome binding site (RBS), 6-histidine tag (6-His) and restriction enzyme cloning sites. The pQE-9 vector was digested with BamHI and XbaI, and the amplified fragment was ligated into the pQE-9 vector maintaining the reading frame initiated at the bacterial RBS. The ligation mixture was then used to transform Escherichia coli strain M15/rep4. Transformants were identified by their ability to grow on Luria-Bertani (LB) plates and ampicillin/kanamycin resistant colonies were selected. Plasmid DNA was isolated and confirmed by restriction analysis. Clones containing the desired constructs were grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 micro-g/ml) and Kan (25 micro-g/ml). The O/N culture was used to inoculate a large culture at a ratio of 1:100-1:250. The cells were grown to an optical density 600 (O.D.(600) of 0.4-0.6. 1 mM of IPTG was then added for inducing protein expression by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells were then harvested by centrifugation, cell debris was removed, and the supernatant containing the polypeptide was purified.(225 pages)

L7 ANSWER 42 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2003-02697 BIOTECHDS
TITLE: Novel PRO polypeptides and nucleic acids encoding the polypeptides, useful for preparing a medicament for the treatment of inflammatory and immune related disorders; vector-mediated gene transfer, expression in host cell and antibody for recombinant protein production and drug screening
AUTHOR: BOTSTEIN D; DESNOYERS L; FERRARA N; FONG S; GAO W; GODDARD A; GURNEY A L; PAN J; ROY M A; STEWART T A; TUMAS D; WATANABE C K; WOOD W I
PATENT ASSIGNEE: GENENTECH INC
PATENT INFO: US 2002098507 25 Jul 2002
APPLICATION INFO: US 2001-33326 27 Dec 2001
PRIORITY INFO: WO 2000-32678 1 Dec 2000; WO 1999-12252 2 Jun 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-673823 [72]
AN 2003-02697 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - An isolated PRO polypeptide (I), having at least 80% identity or scoring at least 80% positives when compared to a sequence (S1) of 278, 830, 125, 325, 437, 487, 310, 1029 or 548 amino acids defined in the specification or to a sequence (S2) encoded by a coding sequence of a DNA deposited under ATCC Accession No. 203538, 203661, 203583, 203657, 203576, 203573, 203553, 203651 and 203537, is new.

DETAILED DESCRIPTION - An isolated PRO polypeptide (I), having at least 80% identity or scoring at least 80% positives when compared to a sequence (S1) of 278, 830, 125, 325, 437, 487, 310, 1029 or 548 amino acids defined in the specification or to a sequence (S2) encoded by a coding sequence of a DNA deposited under ATCC Accession No. 203538, 203661, 203583, 203657, 203576, 203573, 203553, 203651 and 203537, is new. (I) is selected from PRO1800, PRO539, PRO982, PRO1434, PRO1863, PRO1917, PRO1868, PRO3434 and PRO1927. (I) comprises a polypeptide having at least 80% identity or scoring at least 80% positives when compared to S1 or S2, a polypeptide having at least 80% amino acid sequence identity to S1 and lacking its associated signal peptide, or an extracellular domain of S1 with or without its associated signal peptide. INDEPENDENT CLAIMS are also included for the following: (1) an isolated nucleic acid (II) having at least 80% nucleic acid sequence identity to: (a) a nucleotide sequence encoding S1; (b) a nucleotide sequence (S3) comprising 1283, 3121, 662, 1942, 1587, 2387, 3554, 3437 or 2186 nucleotides fully defined in the specification; (c) a nucleotide sequence consisting of the full-length coding sequence of S2 or S3; or (d) a nucleotide sequence encoding S1 or an extracellular domain of S1 with or without its associated signal peptide; (**2**) a vector (III) comprising (II); (3) a host cell (IV) comprising (III); (4) producing a PRO polypeptide (I) comprising culturing (IV); (5) a chimeric molecule (V) comprising (I) fused to a heterologous amino acid sequence; and (6) an antibody (Ab) which specifically binds to (I).

WIDER DISCLOSURE - The following are disclosed: (1) identifying agonists and antagonists of (I); (**2**) agonists and antagonists of (I); (3) a composition comprising (I), or agonists and antagonists of (I);(4) alternatively spliced variants of (I); (5) screening or assaying for identifying molecules that alter modulation of (I) hedgehog signaling; (6) diagnosing to determine whether a particular disorder is modulated by hedgehog signaling; (7) determining the presence of (I) in a sample; (8) diagnosing an inflammatory **disease** in a mammal; (9) a diagnostic kit containing anti-PRO1868 antibody and its carrier; (10) an article of manufacture comprising an active agent that stimulates and inhibits the expression and/or activity of PRO1868 polypeptide; (11) identifying a compound capable of inhibiting the expression and/or activity of PRO1868 polypeptide; (12) oligonucleotide probes for isolating genomic and cDNA nucleotide sequences; (13) fragments of (I) and (II); and (14) immunoconjugates comprising Ab and a cytotoxic agent.

BIOTECHNOLOGY - Preparation: (I) is produced by culturing (IV) under conditions suitable for expression of the PRO polypeptide and recovering the PRO polypeptide from the cell culture (claimed). Preferred Vector: In (III), (II) is operably linked to control sequences recognized by the host cell transformed with the vector. Preferred Cell: (IV) is a Chinese hamster ovary (CHO) cell, Escherichia coli cell or yeast cell. Preferred Molecule: In (V), the heterologous amino acid sequence is an epitope tag sequences or an Fc region of an immunoglobulin. Preferred Antibody: Ab is a monoclonal, humanized or a single-chain antibody.

ACTIVITY - Antiinflammatory; antirheumatic; antiarthritic; immunosuppressive; antianemic; antithyroid; antidiabetic; neuroprotective; hepatotropic; antiinflammatory; virucide; dermatological; antiallergic; antipsoriatic.

MECHANISM OF ACTION - Inhibitor of stimulated T-lymphocyte proliferation; vaccine; gene therapy. Experimental protocols are given, but results are not given.

USE - (I) is useful for the diagnosis and treatment of inflammatory diseases (e.g. inflammatory bowel **disease**, rheumatoid arthritis, Sjogren's syndrome, autoimmune hemolytic anemia, thyroiditis,

diabetes mellitus, multiple sclerosis, hepatitis, contact dermatitis, allergic diseases and psoriasis) and immune related diseases in human. (I) and Ab are useful to prepare medicines and medicaments for treatment of inflammatory and immune related diseases. (I) is also useful for treating kidney diseases. (I) and (II) are useful as molecular weight **markers** for protein **electrophoresis**, and for tissue typing. (II) is useful for chromosome identification. (I) is useful as therapeutic agent. Ab is useful in diagnostic assays for detecting expression of PRO, for the affinity purification of PRO and in the therapeutic treatment of diseases described above.

ADMINISTRATION - Ten ng-100 mg/kg body weight/day, preferably 1 microgram-10 mg/kg/day of (I) is administered through intraperitoneal, intravenous, intracerebral, intramuscular, intraocular, intraarterial, intralesional or topical route.

EXAMPLE - A consensus DNA sequence was assembled relative to other expressed sequence tag (EST) sequences. This consensus sequence was designated DNA30934. Based on the DNA30934 consensus sequence, oligonucleotides were synthesized to identify by polymerase chain reaction (PCR), a cDNA library that contained the sequence of interest, and for use as probes to isolate a clone of the full-length coding sequence for PRO1800. PCR primers 30934.f1 (5'-GCTAATGGATGTCACTGAGG-3') and 30934.r1 (5'-AGAACAATCCTGCTGAAAGCTAG-3') were synthesized. Additionally, a synthetic oligonucleotide hybridization probe was constructed from the consensus DNA30934 sequence comprising a hybridization probe (30934.p1) 5'-GAAACGAGGAGGCGGCTCAGTGGTGATCGTGTCTTCCAT AGCAGCC-3'. RNA for construction of the cDNA libraries was isolated from human fetal liver tissue. DNA sequencing of the clones isolated gave the full-length DNA sequence for PRO1800 (designated as DNA35672-2508) and the derived protein sequence for PRO1800. Clone DNA35672-2508 (comprising 1283 nucleotides fully defined in the specification) contained a single open reading frame with an apparent translational initiation site at nucleotide positions 36-38 and ending at the stop codon at positions 870-872. The predicted polypeptide precursor was 278 amino acids long. The full-length PRO1800 protein had an estimated molecular weight of about 29537 daltons and a pI of about 8.97. Analysis of the full-length PRO180 sequence evidenced the presence of a signal peptide between amino acids 1-15, potential N-glycosylation site between amino acids 183-186, potential N-myristoylation sites between amino acids 43-48, 80-85, 191-196, 213-218 and 272-277, and microbodies C-terminal **targeting** signal between amino acids 276-278.(125 pages)

L7 ANSWER 43 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2003-03721 BIOTECHDS
TITLE: Novel secreted and transmembrane polypeptides and polynucleotides useful for diagnosis and treatment of inflammatory disorders and immune-related diseases, and identifying modulators;
vector-mediated recombinant protein gene transfer and expression in Chinese hamster ovary cell culture, Escherichia coli or yeast cell for use in drug screening and gene therapy
AUTHOR: BOTSTEIN D; DESNOYERS L; FERRARA N; FONG S; GAO W; GODDARD A; GURNEY A L; PAN J; ROY M A; STEWART T A; TUMAS D; WATANABE C K; WOOD W I
PATENT ASSIGNEE: GENENTECH INC
PATENT INFO: US 2002098506 25 Jul 2002
APPLICATION INFO: US 2001-33301 27 Dec 2001
PRIORITY INFO: WO 2000-32678 1 Dec 2000; WO 1999-12252 2 Jun 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-690475 [74]
AN 2003-03721 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Isolated polypeptide (I) having at least 80% amino acid

sequence identity to secreted and transmembrane polypeptides PRO1800, PRO539, PRO982, PRO1434, PRO1863, PRO1917, PRO1868, PRO3434 or PRO1927, is new.

DETAILED DESCRIPTION - Isolated polypeptide (I) having at least 80% amino acid sequence identity to secreted and transmembrane polypeptides PRO1800, PRO539, PRO982, PRO1434, PRO1863, PRO1917, PRO1868, PRO3434 or PRO1927 having a sequence 278, 830, 125, 325, 437, 487, 310, 1029 or 548 amino acids, respectively given in specification, lacking its associated signal peptide, or an extracellular domain of the polypeptide with or without signal peptide. (I) has at least 80% amino acid sequence identity to an amino acid sequence encoded by the full-length coding sequence of the DNA deposited under ATCC accession number 203538, 203661, 203583, 203657, 203576, 203573, 203553, 203651 and 203537. INDEPENDENT CLAIMS are also included for the following: (1) an isolated nucleic acid (II) having at least 80% nucleic acid sequence identity to a nucleotide sequence encoding (I), or a nucleotide sequence consisting of the sequence of 1283, 3121, 662, 1942, 1587, 2387, 3554, 3437 or 2186 base pairs (bp) given in the specification; (2) a vector (III) comprising (II); (3) a host cell (IV) comprising (III); (4) producing PRO polypeptides; (5) a chimeric molecule (V) comprising (I), fused to a heterologous amino acid sequence; and (6) an antibody (VI) which specifically binds to (I).

WIDER DISCLOSURE - Also disclosed are: (1) complement of (II); (2) fragment of (I); (3) agonist or antagonist of PRO polypeptides; (4) composition comprising PRO polypeptides, or agonists or antagonists of PRO polypeptides; (5) variants of PRO polypeptides; (6) immunoconjugates comprising (IV) conjugated to cytotoxic agent; (7) diagnostic kit containing anti-PRO antibody; and (8) oligonucleotide probes for isolating genomic and cDNA nucleotide sequences, derived from (II).

BIOTECHNOLOGY - Preparation: (I) is prepared by culturing (IV), preferably a Chinese hamster ovary (CHO) cell, Escherichia coli, or a yeast cell under conditions suitable for expression of the PRO polypeptide and recovering the PRO polypeptide from the cell culture (claimed). Preferred Vector: (III) is operably linked to control sequences recognized by a host cell transformed with the vector. Preferred Chimeric Molecule: In (V), the heterologous amino acid sequence is an epitope tag sequence or a Fc region of an immunoglobulin. Preferred Antibody: (VI) is a monoclonal, humanized or single chain antibody.

ACTIVITY - Antiinflammatory; Dermatological; Immunosuppressive; Antirheumatic; Antiarthritic; Antianemic; Antithyroid; Antidiabetic; Hepatotropic; Antipsoriatic; Antiallergic; Anti-tumor. Test details are given, but results are not given.

MECHANISM OF ACTION - Gene therapy.

USE - PRO polypeptides are useful for identifying modulators of the polypeptide. PRO1868 useful for the diagnosis and treatment of inflammatory and immune related diseases including systemic lupus erythematosis, rheumatoid arthritis, systemic sclerosis, autoimmune hemolytic anemia, thyroiditis, **diabetes** mellitus, infectious hepatitis, psoriasis, allergic diseases of the lung and graft-versus host **disease**, and tumor. (II) is useful for constructing hybridization probes for mapping the gene that encodes that PRO and for the genetic analysis of individuals with genetic disorders, and for generating transgenic animals which are useful in the development and screening of therapeutically useful reagents. (II) is also useful for gene therapy, chromosome identification, and tissue typing (I) is useful as molecular weight **markers** for protein **electrophoresis** purposes.
The anti-PRO antibodies are useful in diagnostic assays for PRO, e.g., detecting its expression in specific cells, tissues or serum and for affinity purification of PRO.

ADMINISTRATION - Administered by parenteral, oral, topical or by sustained release systems. Dosage not specified.

EXAMPLE - A consensus DNA sequence was assembled relative to other expressed sequence tag (EST) sequences using phrap. This consensus sequence was designated DNA30934. Based on the DNA30934 consensus

sequences, oligonucleotides were synthesized to identify by polymerase chain reaction (PCR) a cDNA library that contained the sequence of interest, and for use as probes to isolate a clone of the full-length coding sequence for PRO1800. PCR primers were synthesized, the forward PCR primer (30934.f1) 5'-GCATAATGGATGTCACTGAGG-3', reverse PCR primer (30934.r1) 5'-AGAACAATCCTGCTGAAAGCTAG-3'. Additionally, a synthetic oligonucleotide hybridization probe was constructed from the consensus DNA30934 sequence which had the nucleotide sequence 5'-GAAACGAGGAGGCGGCTCAGTGGTGATCGTGTCTTCCATAGCAGCC-3'. RNA for construction of the cDNA libraries was isolated from human fetal liver tissue. DNA sequencing of the clones isolated gave the full-length DNA sequence for PRO1800 and the derived protein sequence for PRO1800. The predicted polypeptide precursor was 278 amino acids long and the DNA sequence comprised 1283 base pairs (bp) given in the specification. (125 pages)

L7 ANSWER 44 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-14218 BIOTECHDS
TITLE: Making transgenic avian lacking expression of endogenous immunoglobulin by inactivating endogenous immunoglobulin heavy chain locus in a avian cell and generating an avian from the cell;
fowl, turkey, duck, goose or quail transgenic animal construction and monoclonal antibody for animal breeding
AUTHOR: SINGH S; DIAS P
PATENT ASSIGNEE: SINGH S; DIAS P
PATENT INFO: US 2002028488 7 Mar 2002
APPLICATION INFO: US 2000-884579 19 Jun 2000
PRIORITY INFO: US 2001-884579 18 Jun 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-280153 [32]
AN 2002-14218 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Making (M1) a transgenic avian (I) lacking expression of endogenous immunoglobulin (Ig) by inactivating at least one endogenous heavy chain Ig locus in a avian cell, generating an avian from the cell, and optionally breeding a avian to obtain (I) lacking expression of endogenous Igs.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) making (M2) a chimeric or xenogenic antibody, by immunizing (I) of M1 with an antigen, harvesting serum or obtaining at least one egg from the transgenic avian, and isolating one chimeric antibody or one exogenous or xenogenic antibody from the serum or egg; (**2**) making (M3) a chimeric or xenogenic monoclonal antibody by immunizing (I) of M1 with an antigen, harvesting B cells from (I), immortalizing the B cell, and isolating monoclonal antibody from the culture medium of the B cells; and (3) an antibody made by M2 or M3.

WIDER DISCLOSURE - Also disclosed are: (1) isolation of antibody producing cells from (I) that has been immunized with an desired antigen, the cells can be immortalized for the production of antibody in culture, the immortalized cells can be used for the isolation of cDNAs encoding immunoglobulin heavy and light chains or their portions; (**2**) generation of genomic DNA deletions or gene disruptions in avian cells, which provides the use of a replacement-type **targeting** construct to delete fragments of genomic DNA by gene **targeting**; and (3) production of polyclonal human antibodies from avian serum or eggs.

BIOTECHNOLOGY - Preferred Method: M1 further comprises introducing a portion of an exogenous Ig locus into the avian cell, where the portion preferably comprises a portion of one heavy chain constant region which is a human heavy chain constant region which is from VH, DH, JH and CH regions; inactivating and introducing one endogenous Ig light chain locus preferably of human which comprises VL, JL and CL regions in the cell of avian which is chicken, turkey, duck, goose or quail. M3 utilized for

making xenogenic monoclonal antibody, further comprises isolating at least one nucleic acid molecule by cDNA encoding at least a portion of an immunoglobulin from the immortalized B cells, introducing at least one nucleic acid molecule comprising cDNA encoding at least a portion of an immunoglobulin into at least one other cell; culturing at least one other cell under conditions that promote protein synthesis, and isolating at least one antibody from the culture medium of the at least one other cell, which is prokaryotic, fungal, avian or mammalian cell.

USE - M1 is useful for making (I) lacking expression of endogenous Ig, M2 is useful for making a chimeric/xenogenic antibody, and M3 is useful for making a chimeric or xenogenic monoclonal antibody (claimed), where (I) obtained by M1 can be immunized against an antigen and screened for the production of antibodies that bind to the antigen, and the antibodies can be used as a source of antibody that can be purified from eggs or from serum, and for isolation of B-cells that can be immortalized, screened for the production of antibodies that bind the antigen, and for the isolation of B-lymphocytes that can be used as a source of mRNA of cloning cDNAs that can encode human immunoglobulin light chains and/or heavy chains. The immortalized cells of M3 can also be used for isolation of the genes encoding the immunoglobulin or analog and the genes can optionally be subjected to mutation by in vitro mutagenesis or other mutagenizing techniques. The cells and (I) which contain the genomic deletions may be used to study gene structure and functions or biochemical processes such as for e.g. protein production or inhibition. (I) may be used as a source of cells, organs, or tissues or to provide model systems for human **disease**, such as for e.g. immune system disorders, or **disease** such as type I **diabetes** and multiple sclerosis, that may have an autoimmune component.

ADVANTAGE - The advantage of avian system is that the zygote is highly accessible to the researcher as it develops external to the female organism.

EXAMPLE - A 4.5 Kb fragment containing the chicken heavy chain J genes and flanking sequences, was polymerase chain reaction (PCR) amplified from a White Leghorn chicken strain genomic library containing Eco RI cloning sites in the PCR primers and inserted into ECoRI-digested pUC19 plasmid (pchkJH) (for chicken heavy gene complex). An 1150 bp Xho I-Bam HI fragment, containing a neomycin-resistance gene driven by the herpes simplex virus thymidine kinase gene (HSV-tk) promoter and a polyoma enhancer was isolated from pMCINeo. A synthetic adaptor was added onto this fragment to convert the Bam HI end into a ScaI end and the resulting fragment was joined to the Xho I-Sca I digested PchkJH to form the inactivation vector in which the heavy chain J genes were excised, and the 5' to 3' orientation of the neomycin and the heavy chains promoters was identical. This plasmid was linearized by Nde I digestion before transfection into embryonic stem (ES) cells. The ES cells were isolated from blastodermal cells, maintained and amplified in vitro. The chicken ES cells (CES) derived as above as transfected with JH inactivating vector were selected. ES colonies remaining 10-14 days after transfection were picked with drawn out capillary pipettes for analysis using polymerase chain reaction (PCR), where one priming oligonucleotide corresponded to a region 650 bases 3' of the start codon of the neomycin resistance gene and the other priming oligonucleotide corresponded to sequences located in the human heavy chain gene that are outside the region of homology included in the **targeting** vector. Each reaction mix was electrophoresed on agarose gels and transferred to nylon membranes. Filters were probed with a 32P-labeled fragment of the J-C region. Because the PCR primers employed with only amplify a segment of DNA in which the DNA neomycin-resistance gene was physically linked to PCR products that hybridize to the probe, hybridizing PCR products of the expected size were derived from loci in which the neomycin gene had homologously recombined into the J region of the heavy chain locus, thereby inactivating the locus.(31 pages)

L7 ANSWER 45 OF 150 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:977582 CAPLUS
DOCUMENT NUMBER: 138:37450
TITLE: Ras-MEK-ERK1/**2** signaling pathway in the production of inflammatory and neuropathic pain and uses for analgesic screening
INVENTOR(S): Levine, Jon David; Messing, Robert O.
PATENT ASSIGNEE(S): The Regents of the University of California, USA
SOURCE: PCT Int. Appl., 134 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002102232	A2	20021227	WO 2002-US19107	20020614
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
US 2003008807	A1	20030109	US 2002-173332	20020614

PRIORITY APPLN. INFO.: US 2001-298491P P 20010614
AB This invention pertains to the discovery of a novel pathway that mediates hyperalgesia, neuropathic pain, and inflammatory pain. This pathway is a third independent pathway that involves activation of extracellular signal-regulated kinases (ERKs) 1 and **2**. The pathway comprises a Ras-MEK-ERK1/**2** cascade that acts independent of PKA or PKC.epsilon. as a novel signaling pathway for the prodn. of inflammatory (and neuropathic) pain. This pathway presents numerous **targets** for a new class of analgesic agents.

L7 ANSWER 46 OF 150 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:964606 CAPLUS
DOCUMENT NUMBER: 138:35730
TITLE: Mitochondrial protein **targets** for drug screening and therapeutic intervention identified using mass spectrometry
INVENTOR(S): Gibson, Bradford W.; Ghosh, Soumitra S.; Davis, Robert E.
PATENT ASSIGNEE(S): Mitokor, USA; The Regents of the University of California
SOURCE: PCT Int. Appl., 134 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002101356	A2	20021219	WO 2002-US18484	20020610
WO 2002101356	A3	20030227		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ,				

UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
PRIORITY APPLN. INFO.: US 2001-296867P P 20010608
AB The invention concerns mitochondrial **targets** for drug screening assays and for therapeutic intervention in the treatment of diseases assocd. with altered mitochondrial function are provided by generating a high-resoln. (**2**-D) map of mitochondrial proteins, and then isolating at least one protein and subjecting it to mass spectrometric anal., including MALDI-TOF MS. Complete amino acid sequences [SEQ ID NOS:1-8] of polypeptides that comprise the human mitochondrial proteome are provided, using protein and peptide fractions of biol. samples derived from mitochondrial cybrid (cytoplasmic hybrid) cell lines, to identify previously unrecognized mitochondrial mol. components, including modified polypeptides that exhibit structural and/or functional alterations in diseases assocd. with altered mitochondrial function.

L7 ANSWER 47 OF 150 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:696163 CAPLUS
DOCUMENT NUMBER: 137:211910
TITLE: Method for the development of panels of genes for diagnosis and therapy based on the expression and methylation status of the genes
INVENTOR(S): Olek, Alexander; Berlin, Kurt
PATENT ASSIGNEE(S): Epigenomics AG, Germany
SOURCE: PCT Int. Appl., 92 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002070742	A1	20020912	WO 2002-EP2255	20020301
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
US 2002137086	A1	20020926	US 2002-87466	20020301

PRIORITY APPLN. INFO.: US 2001-272549P P 20010301
AB The invention concerns a method for the development of gene panels for diagnostic and therapeutic purposes based on the expression and methylation status of specific genes. The invention further concerns gene panels developed using the method of the present invention and their uses.
REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 48 OF 150 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:449914 CAPLUS
DOCUMENT NUMBER: 137:28999
TITLE: Method for the determination of at least one functional polymorphism in the nucleotide sequence of a preselected candidate gene and its applications
INVENTOR(S): Escary, Jean-Louis
PATENT ASSIGNEE(S): Genodyssee, Fr.
SOURCE: PCT Int. Appl., 64 pp.

CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002046459	A2	20020613	WO 2001-EP15427	20011206
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
FR 2817559	A1	20020607	FR 2000-15838	20001206
AU 2002019230	A5	20020618	AU 2002-19230	20011206
US 2002155467	A1	20021024	US 2001-10749	20011206

PRIORITY APPLN. INFO.: FR 2000-15838 A 20001206
WO 2001-EP15427 W 20011206

AB The present invention concerns a method for detg. at least one functional SNP in a gene, comprising preselecting a candidate gene, providing a sample population comprising a significant no. of individuals chosen substantially at random from the general population, isolating from each individual of the sample population at least one fragment of the nucleotide sequence of the preselected candidate gene, identifying at least one SNP in at least one fragment and detg. the functionality of said SNP(s). The present invention also concerns applications of this method.

L7 ANSWER 49 OF 150 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:220406 CAPLUS
DOCUMENT NUMBER: 136:244020
TITLE: Non-genetic based protein **disease markers**
INVENTOR(S): Rembert, Pieper; Taylor, John, Jr.; Steiner, Sandra; Anderson, N. Leigh; Myers, Timothy
PATENT ASSIGNEE(S): Large Scale Proteomics Corporation, USA
SOURCE: PCT Int. Appl., 63 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002022165	A1	20020321	WO 2001-US28268	20010912
W: AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EC, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
US 2002072492	A1	20020613	US 2001-886271	20010622
AU 2001088973	A5	20020326	AU 2001-88973	20010912

PRIORITY APPLN. INFO.: US 2000-660242 A 20000912
US 2001-886271 A 20010622
WO 2001-US28268 W 20010912

AB The invention concerns protein **disease markers** for **obesity**, **osteoporosis**, **diabetes**, **osteoarthritis** and **hypertension** are disclosed. These **markers** are not inherited or of genetic origin as they were not found in identical twins of the affected individual. Methods and uses for diagnostic, therapeutic and drug discovery are disclosed.

REFERENCE COUNT: 1 THERE ARE 1 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 50 OF 150 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:450337 CAPLUS
DOCUMENT NUMBER: 137:2745
TITLE: Non-genetic based protein **disease markers**
INVENTOR(S): Myers, Timothy G.; Pieper, Rembert; Taylor, John; Steiner, Sandra; Anderson, N. Leigh
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 26 pp., Cont.-in-part of U. S. Ser. No. 660,242.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2002072492	A1	20020613	US 2001-886271	20010622
WO 2002022165	A1	20020321	WO 2001-US28268	20010912
W: AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EC, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
AU 2001088973	A5	20020326	AU 2001-88973	20010912

PRIORITY APPLN. INFO.:
US 2000-660242 A2 20000912
US 2001-886271 A 20010622
WO 2001-US28268 W 20010912

AB The invention concerns protein **disease markers** for **obesity**, **osteoporosis**, **diabetes**, **osteoarthritis** and **hypertension**. These **markers** are not inherited or of genetic origin as they were not found in identical twins of the affected individual. Methods and uses for diagnostic, therapeutic and drug discovery are disclosed.

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'L999-998' IS NOT A VALID FORMAT
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L7 ANSWER 51 OF 150 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:336239 BIOSIS
DOCUMENT NUMBER: PREV200200336239
TITLE: A novel allelic variant of the human TSG-6 gene encoding an amino acid difference in the CUB module. Chromosomal localization, frequency analysis, modeling, and expression.
AUTHOR(S): Nentwich, Hilke A.; Mustafa, Zehra; Rugg, Marilyn S.; Marsden, Brian D.; Cordell, Martin R.; Mahoney, David J.; Jenkins, Suzanne C.; Dowling, Barbara; Fries, Erik; Milner, Caroline M.; Loughlin, John; Day, Anthony J. (1)
CORPORATE SOURCE: (1) Medical Research Council Immunochemistry Unit, University of Oxford, South Parks Rd., Oxford, OX1 3QU: tony.day@bioch.ox.ac.uk UK
SOURCE: Journal of Biological Chemistry, (May 3, 2002) Vol. 277, No. 18, pp. 15354-15362. http://www.jbc.org/. print. ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Tumor necrosis factor-stimulated gene-6 (TSG-6) encodes a 35-kDa protein, which is comprised of contiguous Link and CUB modules. TSG-6 protein has been detected in the articular joints of osteoarthritis (OA) patients, with little or no constitutive expression in normal adult tissues. It interacts with components of cartilage matrix (e.g. hyaluronan and aggrecan) and thus may be involved in extracellular remodeling during joint disease. In addition, TSG-6 has been found to have anti-inflammatory properties in models of acute and chronic inflammation. Here we have mapped the human TSG-6 gene to 2q23.3, a region of chromosome 2 linked with OA. A single nucleotide polymorphism was identified that involves a non-synonymous G fwdarw A transition at nucleotide 431 of the TSG-6 coding sequence, resulting in an Arg to Gln alteration in the CUB module (at residue 144 in the preprotein). Molecular modeling of the CUB domain indicated that this amino acid change might lead to functional differences. Typing of 400 OA cases and 400 controls revealed that the A431 variant identified here is the major TSG-6 allele in Caucasians (with over 75% being A431 homozygotes) but that this polymorphism is not a marker for OA susceptibility in the patients we have studied. Expression of the Arg144 and Gln144 allotypes in Drosophila Schneider 2 cells, and functional characterization, showed that there were no significant differences in the ability of these full-length proteins to bind hyaluronan or form a stable complex with inter-alpha-inhibitor.

L7 ANSWER 52 OF 150 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2003:113563 BIOSIS
DOCUMENT NUMBER: PREV200300113563

TITLE: Capillary zone electrophoresis study of cyclodextrin: Lipoic acid host-guest interaction.
AUTHOR(S): Trentin, Marco; Carofiglio, Tommaso (1); Fornasier, Roberto; Tonellato, Umberto
CORPORATE SOURCE: (1) Dipartimento di Chimica Inorganica, Metallorganica ed Analitica, Centro Meccanismi Reazioni Organiche, C.N.R., Universita di Padova, Via Marzolo 1, I-35131, Padova, Italy: tommaso.carofiglio@unipd.it, roberto.fornasier@unipd.it Italy
SOURCE: Electrophoresis, (December 2002, 2002) Vol. 23, No. 24, pp. 4117-4122. print.
ISSN: 0173-0835.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Lipoic acid is a naturally occurring compound which is being widely investigated for its therapeutic effects in the treatment or prevention of a variety of diseases associated with oxidative injury, particularly diabetes. The diversity of therapeutic applications of lipoic acid requires an appropriate formulation to control its bioavailability, site-targeting delivery and to overcome its inherent chemical instability. In this regard, cyclodextrins (CDs) are ideally suitable due to their well-documented ability to include in their cavity proper guest molecules and protect them from physical or chemical damages. Lipoic acid forms 1:1 inclusion complexes with betaCD as shown in a previous report of an extended investigation that also indicated the suitability of capillary zone electrophoresis (CZE) for the study of such host-guest interactions. In view of these possible applications, we extended the CZE analysis to determine the strength of binding, in a pH 9 phosphate buffer, of lipoic acid with other CD derivatives such as alphaCD, gammaCD and the alkylated derivatives of betaCD, namely (2-hydroxypropyl)-beta-CD (HPbetaCD), and heptakis (2,3,6-tri-O-methyl)-beta-CD (TMbetaCD). Once established that the easily available betaCD is the most suitable receptor for lipoic acid, we set up and here describe a simple and reliable procedure for the quantitative determination of lipoic acid in commercial dietary supplement tablets containing also other active substances and excipients.

L7 ANSWER 53 OF 150 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 1
ACCESSION NUMBER: 2002:826115 CAPLUS
DOCUMENT NUMBER: 138:71182
TITLE: Mass spectrometric proteome analyses of synovial fluids and plasmas from patients suffering from rheumatoid arthritis and comparison to reactive arthritis or osteoarthritis
AUTHOR(S): Sinz, Andrea; Bantscheff, Marcus; Mikkat, Stefan; Ringel, Bruno; Drynda, Susanne; Kekow, Jorn; Thiesen,

Hans-Jurgen; Glocker, Michael O.
CORPORATE SOURCE: Proteome Center Rostock, Medical Faculty, University of Rostock, Rostock, D-18059, Germany
SOURCE: Electrophoresis (2002), 23(19), 3445-3456
CODEN: ELCTDN; ISSN: 0173-0835
PUBLISHER: Wiley-VCH Verlag GmbH & Co. KGaA
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Differential proteome anal. is used to study body fluids from patients suffering from rheumatoid arthritis (RA), reactive arthritis (reaA) or osteoarthritis (OA). Mass spectrometric structure characterization of gel-sepd. proteins provided a detailed view of the protein-processing events that lead to distinct protein species present in the resp. body fluids. Fibrinogen .beta.-chain degrdn. products, presumably plasmin-derived, appeared solely in synovial fluids (SF) from both patient collectives, calgranulin B (MRP14) was exclusively identified in SF samples derived from 5 out of 6 patients suffering from RA. Calgranulin B was not obsd. in synovial fluids from OA patients, nor in plasmas from either patient group. In all cases where calgranulin B was detected, calgranulin C was identified as well. Serum amyloid A protein spots were detd. in plasmas and synovial fluids from patients with RA, but not in patients with OA. In addn. to disease-relevant differences, interindividual differences in haptoglobin patterns of the patients under investigation were obsd. Hence, in-depth proteome anal. of body fluids has proven effective for identification of multiple mol. markers and detn. of assocd. protein structure modifications, that are thought to play a role for specifically detg. a defined pathol. state of diseased joints.
REFERENCE COUNT: 42 THERE ARE 42 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 54 OF 150 MEDLINE
ACCESSION NUMBER: 2002713595 MEDLINE
DOCUMENT NUMBER: 22363716 PubMed ID: 12475459
TITLE: Haptoglobin phenotype is an independent risk factor for cardiovascular disease in individuals with diabetes: The Strong Heart Study.
AUTHOR: Levy Andrew P; Hochberg Irit; Jablonski Kathleen; Resnick Helaine E; Lee Elisa T; Best Lyle; Howard Barbara V
CORPORATE SOURCE: Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel. (The Strong Heart Study). alevy@tx.technion.ac.il
CONTRACT NUMBER: 41652 (NHLBI)
41654 (NHLBI)
R01 HL-66195

U01-HL-41642
SOURCE: JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY, (2002 Dec 4)
40 (11) 1984-90.
Journal code: 8301365. ISSN: 0735-1097.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200301
ENTRY DATE: Entered STN: 20021217
Last Updated on STN: 20030116
Entered Medline: 20030115

AB OBJECTIVES: The goal of this study was to determine if the haptoglobin phenotype was predictive of cardiovascular disease (CVD) in diabetic mellitus (DM). BACKGROUND: Cardiovascular disease is the most frequent, severe, and costly complication of type 2 DM. There are clear geographic and ethnic differences in the risk of CVD among diabetic patients that cannot be fully explained by differences in conventional CVD risk factors. We have demonstrated that a functional allelic polymorphism in the haptoglobin gene acts as a major determinant of susceptibility for the development of diabetic microvascular complications. METHODS: We sought to determine if this paradigm concerning the haptoglobin gene could be extended to CVD in DM. We tested this hypothesis in a case-control sample from the Strong Heart study, a population-based longitudinal study of CVD in American Indians. Haptoglobin phenotype was determined by polyacrylamide gel electrophoresis in 206 CVD cases and 206 matched controls age 45 to 74 years. Median follow-up was six years. RESULTS: In multivariate analyses controlling for conventional CVD risk factors, haptoglobin phenotype was a highly statistically significant, independent predictor of CVD in DM. The odds ratio of having CVD in DM with the haptoglobin 2-2 phenotype was 5.0 times greater than in DM with the haptoglobin 1-1 phenotype ($p = 0.002$). An intermediate risk of CVD was associated with the haptoglobin 2-1 phenotype. CONCLUSIONS: This study suggests that determination of haptoglobin phenotype may contribute to the algorithm used in CVD risk stratification, and in evaluation of new therapies to prevent CVD in the diabetic patient.

L7 ANSWER 55 OF 150 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 2002620307 MEDLINE
DOCUMENT NUMBER: 22265090 PubMed ID: 12377213
TITLE: Specific monoclonal antibodies against the surface of rat islet beta cells.
AUTHOR: Konidaris Constantinos; Simonson Will; Michelsen Birgitte; Papadopoulos George K

CORPORATE SOURCE: Laboratory of Biological Chemistry, University of Ioannina Medical School, GR45100, Ioannina, Greece.
SOURCE: CELL BIOLOGY INTERNATIONAL, (2002) 26 (9) 817-28.
Journal code: 9307129. ISSN: 1065-6995.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200303
ENTRY DATE: Entered STN: 20021017
Last Updated on STN: 20030313
Entered Medline: 20030312

AB Type 1 diabetes arises from the autoimmune destruction of islet beta cells, with the participation of both arms of the immune system. To better characterize the beta cell membrane, we have raised monoclonal antibodies to the surface of the INS-1 insulinoma cell line. Twenty-two such antibodies were produced, 21 of the IgG class, all reactive to different cell membrane proteins from INS-1 and neonatal islet cells, yielding identical electrophoresis patterns, with molecular weights mainly between 45 and 60 kD. We have focused on three such antibodies that recognize different protein targets, and are specific for islet beta cells. The target protein of antibody AA4, also found on monkey islets, is expressed at significantly higher levels on beta cells (55.8 vs 30.6% of cells, plus 3-4 fold increase in average fluorescence intensity per cell) when neonatal rat islet cells are incubated with high (16 mM vs 3mM) glucose concentrations. Further identification of the target antigens is in progress and is expected to shed more light on the properties of beta cell membrane proteins, and their probable participation in various disease processes.

L7 ANSWER 56 OF 150 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 2002269044 EMBASE
TITLE: Impaired neutrophil actin assembly causes persistent CD11b expression and reduced primary granule exocytosis in Type II diabetes.
AUTHOR: Advani A.; Marshall S.M.; Thomas T.H.
CORPORATE SOURCE: Dr. T.H. Thomas, Department of Medicine, Medical School, University of Newcastle Upon Tyne, Framlington Place, Newcastle Upon Tyne NE2 4HH, United Kingdom.
T.H.Thomas@ncl.ac.uk
SOURCE: Diabetologia, (2002) 45/5 (719-727).
Refs: 36
ISSN: 0012-186X CODEN: DBTGAJ
COUNTRY: Germany
DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 003 Endocrinology
005 General Pathology and Pathological Anatomy
006 Internal Medicine
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Aims/hypothesis. Neutrophil dysfunction has a role in the pathogenesis of complications in Type II (non-insulin-dependent) diabetes mellitus. Neutrophils adhere through expression of the .beta.(2)integrin CD11b/CD18 which closely associates with the actin cytoskeleton. The aim of this study was to investigate the effect of actin polymerisation on CD11b expression and exocytosis of the primary granule marker CD69 in neutrophils from patients with Type II diabetes. Methods. Neutrophils were activated with fMLP or PMA, actin polymerisation was inhibited with cytochalasin D. Cells were stained for CD11b and CD69 expression and intracellular F-actin was measured with phalloidin-FITC. Cellular fluorescence was measured by flow cytometry. Actin content of Triton X-100 fractions of cells was measured by SDS-PAGE and Coomassie blue staining. Results. PMA caused an increase in neutrophil F-actin that was greater in control subjects than in patients with Type II diabetes (50.8% vs 33.4%, $p<0.001$) and correlated with actin integrated optical density (IOD) by SDS-PAGE ($r=0.74$, $p=0.01$). Loss of CD11b from cell surfaces only occurred in neutrophils with high F-actin. The proportion of cells losing CD11b was lower in patients than in control subjects (23.1% vs 37.5%, $p<0.001$) and lowest in patients with additional cardiovascular risk markers (20.1% vs 27.7%; $p<0.05$). Cytochalasin D prevented loss of CD11b ($p<0.001$). CD69 expression was reduced in patients with Type II diabetes (22.6% vs 36.4%, $p<0.001$) and correlated with F-actin content ($r=0.78$, $p<0.0001$). Conclusion/interpretation. In Type II diabetes impaired neutrophil actin polymerisation leads to persistent expression of CD11b and reduced exocytosis of primary granules and could contribute to the pathogenesis of diabetic complications.

L7 ANSWER 57 OF 150 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 2002243121 MEDLINE
DOCUMENT NUMBER: 21977325 PubMed ID: 11980626
TITLE: Homozygous combination of calpain 10 gene haplotypes is associated with type 2 diabetes mellitus in a Polish population.
AUTHOR: Malecki Maciej T; Moczulski Dariusz K; Klupa Tomasz; Wanic Krzysztof; Cyganek Katarzyna; Frey Jakub; Sieradzki Jacek
CORPORATE SOURCE: Department of Metabolic Diseases, Medical College, Jagiellonian University, 15 Kopernika Street, 31-501 Krakow, Poland.. mmalecki@cm-uj.krakow.pl
CONTRACT NUMBER: 1 R03 TW01315-01 (FIC)

SOURCE: EUROPEAN JOURNAL OF ENDOCRINOLOGY, (2002 May) 146 (5)
695-9.
Journal code: 9423848. ISSN: 0804-4643.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200206
ENTRY DATE: Entered STN: 20020501
Last Updated on STN: 20020623
Entered Medline: 20020621

AB OBJECTIVE: The polymorphisms of two genes have recently been associated with complex forms of type 2 diabetes mellitus (T2DM): calpain 10 and peroxisome proliferator-activated receptor-gamma (PPARgamma). Calpain 10 is a member of a large family of intracellular proteases. It was shown in Mexican-Americans and other populations that variants of three single nucleotide polymorphisms (SNPs), -43, -19, and -63, of this ubiquitously expressed protein influence susceptibility to T2DM. However, substantial differences were shown between ethnic groups in at risk alleles and haplotypes as well as in their attributable risk. Thus, it is important to determine the role of calpain 10 in various populations. AIM: To examine the role of calpain 10 SNPs -43, -19, and -63 in genetic susceptibility to T2DM in a Polish population. METHODS: Overall, 377 individuals were examined: 229 T2DM patients and 148 control individuals. The groups were genotyped for calpain 10 SNP-43, SNP-19, and SNP-63. SNP-19 was examined by electrophoresis of the PCR product on agarose gel by size, while the restriction fragment length polymorphism (RFLP) method was used for the two other markers. Differences in allele, genotype, haplotype, and haplotype combination distribution between the groups were examined by chi(2) test. RESULTS: Distributions of alleles, genotypes, and haplotypes at three loci defined by examined SNPs were not significantly different between the groups. However, the homozygote combination of 121 haplotype was more prevalent in the T2DM group than in the controls (17.9% vs 10.1%, P=0.039). No difference was observed in the 112/121 haplotype distribution. This heterozygous haplotype combination was associated with increased risk of T2DM in several populations. CONCLUSION: The results of our study suggest the association of calpain 10 121/121 haplotype combination created by SNPs -43, -19, and -63 with T2DM in a Polish population. However, we were not able to confirm the previously described role of the heterozygous 112/121 haplotype combination in susceptibility to T2DM.

L7 ANSWER 58 OF 150 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 2002279640 EMBASE

TITLE: Association analysis of genotypic frequencies of matrilin-1 gene in patients with osteoarthritis.
AUTHOR: Strusberg I.; Sembaj A.; Tabares S.; Strusberg A.M.; del Castillo I.; Villamar M.; Moreno Barral J.
CORPORATE SOURCE: I. Strusberg, Av. E. Olmos 247, Cordoba, Argentina. jmoreno@biomed.uncor.edu
SOURCE: Clinical and Experimental Rheumatology, (2002) 20/4 (543-545).
Refs: 15
ISSN: 0392-856X CODEN: CERHDP
COUNTRY: Italy
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 005 General Pathology and Pathological Anatomy
022 Human Genetics
031 Arthritis and Rheumatism
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Objective. It has been suggested that genotypic variation in the gene which encodes the matrilin-1 (MATN-1) protein may be involved in the development of hip osteoarthritis (OA). We compared genotype frequencies of the MATN-1 gene (1p35) in patients with OA and controls to determine if there is any association between the MATN-1 genotype and OA. Methods. 73 OA patients and 53 controls from a rheumatology ambulatory center and a university hospital were studied. They were unrelated subjects. Controls were free of clinical OA. OA was defined according to the American College of Rheumatology criteria. The MATN-1 microsatellite in the 3' untranslated region was amplified by PCR. The size of the amplification products was determined by capilar electrophoresis in a DNA Genetic Analizer. Genotypic distribution was compared by the .CHI.(2) test. Results. We identified 4 alleles according to their basepair (bp) length: A1 = 110 bp; A2 = 108 bp; A3 = 106 bp and A6 = 104 pb. Six genotypes were found, with an observed heterozygosity of 0.48. The most frequent genotype in OA and controls was A1/A1 (43.8% and 43.4%, respectively). No significant difference in genotype distribution was found between OA - even when discriminating by the affected joint - and controls. Conclusion. We did not find any difference in the MATN-1 genotype distribution in OA patients and controls. To our knowledge, this would be the first time a MATN-1 allele of 104 bp (A6) has been identified. These results do not support a role of the MATN-1 genotypes in the ocurrence of clinical OA.

L7 ANSWER 59 OF 150 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:563868 BIOSIS
DOCUMENT NUMBER: PREV200200563868
TITLE: Effect of rosiglitazone on the differential expression of

diabetes-associated proteins in pancreatic islets of C57BI/6 lep/lep mice.

AUTHOR(S): Sanchez, Jean-Charles (1); Converset, Veronique; Nolan, Anna; Schmid, Gerhard; Wang, Steven; Heller, Manfred; Semnitt, Matthew V.; Hochstrasser, Denis F.; Cawthorne, Michael A.

CORPORATE SOURCE: (1) Geneva Proteomics Center, Central Clinical Chemistry Laboratory, Geneva University Hospital, 24 Rue Micheli-du-Crest, 1211, Geneva 14: sanchez@dim.hcuge.ch Switzerland

SOURCE: Molecular & Cellular Proteomics, (July, 2002) Vol. 1, No. 7, pp. 509-516. http://www.mcponline.org. print. ISSN: 1535-9476.

DOCUMENT TYPE: Article

LANGUAGE: English

AB The insulin sensitizer drug, rosiglitazone, has been shown to have a protective effect on pancreatic islet cell structure and function in animal models of type 2 diabetes. The identification of new molecular targets associated both with islet cell dysfunction and protection is a crucial research goal. In the present study, a proteomics approach has been used to identify such targets. Obese C57BI/6J lep/lep mice and lean littermates were given the insulin sensitizer drug BRL49653, rosiglitazone. It normalized the impaired glucose tolerance in lep/lep mice but had no significant effect on glucose tolerance in the lean mice. Pancreatic islet polypeptides were arrayed by a two-dimensional gel electrophoresis system that separated more than 2500 individual spots. Three overexpressed and six underexpressed proteins were significant ($p<0.05$) between lep/lep and lean mice, and four were modulated significantly ($p<0.05$) by the rosiglitazone treatment of the obese mice. The identity of these differentially expressed proteins was made using mass spectrometric analysis and provided evidence that differential expression of actin-binding proteins may be an important aspect of defective islet function. Rosiglitazone increased carboxypeptidase B expression in both lep/lep and normal mice suggesting that this might be an independent effect of rosiglitazone that contributes to improved insulin processing.

L7 ANSWER 60 OF 150 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 2003:14111 BIOSIS

DOCUMENT NUMBER: PREV200300014111

TITLE: Evaluation of a Apo-1/Fas promoter polymorphism in Korean stroke patients.

AUTHOR(S): Seo, Jung-Chul; Han, Sang-Won; Yin, Chang-Sik; Koh, Hyung-Kyun; Kim, Chang-Hwan; Kim, Ee-Hwa; Leem, Kang-Hyun;

Lee, Hyang-Sook; Park, Hi-Joon; Kim, Soon-Ae; Choe, Bong-Keun; Lee, Hee-Jae; Yim, Sung-Vin; Kim, Chang-Ju; Chung, Joo-Ho (1)

CORPORATE SOURCE: (1) College of Medicine, Kohwang Medical Research Institute, Kyung-Hee University, Seoul, 130-701, South Korea: acumox@hanmail.net South Korea

SOURCE: Experimental & Molecular Medicine, (September 30 2002) Vol. 34, No. 4, pp. 294-298. print.
ISSN: 1226-3613.

DOCUMENT TYPE: Article

LANGUAGE: English

AB Apoptosis has been implicated in the pathogenesis of neurodegenerative diseases such as stroke and Alzheimer's disease. Apo-1/Fas gene is one of the mediators of apoptosis in stroke. Mval polymorphism is the first polymorphic marker identified in the Apo-1/Fas gene promoter, which was typed by PCR and followed by Mval digestion and gel electrophoresis. DNA isolated from peripheral blood collected from 91 stroke patients and 103 healthy blood donors was used for genotypes of GG, GA and AA by sequence specific primer PCR. Mval polymorphism was examined based on Fas gene promotor region by restriction fragment length polymorphism (RFLP). The Fas-GG genotype was the least frequent in patients with stroke and healthy controls (P=0.57). In normal Korean controls the Mval polymorphism GA, AA and GG were 48.6%, 34.9% and 16.5%. In stroke patients were 56.2%, 29.6% and 14.2% respectively. And the allelic frequencies of Mval*2 (G) allele were less frequent than Mval*1 (A) allele in patients with stroke and healthy controls (P=0.76). In normal Korean controls Mval*1 (A) and Mval* 2 (G) alleles were 59.2% and 40.8%. In stroke patients were 57.6% and 42.4%, respectively. Our results, pending confirmation in a larger study, indicate that the Fas genotype may not appear to be a risk factor for stroke in Korean stroke patients.

L7 ANSWER 61 OF 150 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

DUPLICATE 4

ACCESSION NUMBER: 2003:72025 BIOSIS

DOCUMENT NUMBER: PREV200300072025

TITLE: LDL-cholesterol/apolipoprotein B ratio is a good predictor of LDL phenotype B in type 2 diabetes.

AUTHOR(S): Wagner, A. M.; Jorba, O.; Rigla, M.; Alonso, E.; Ordonez-Llanos, J.; Perez, A. (1)

CORPORATE SOURCE: (1) Endocrinology and Nutrition Department, Hospital de Sant Pau, Universitat Autonoma, Antonio M. Claret 167, E-08025, Barcelona, Spain Spain

SOURCE: Acta Diabetologica, (December 2002, 2002) Vol. 39, No. 4, pp. 215-220. print.

ISSN: 0940-5429.
DOCUMENT TYPE: Article
LANGUAGE: English
AB LDL phenotype B is a component of diabetic dyslipidaemia, but its diagnosis is cumbersome. Our aim was to find easily available markers of phenotype B in a group of type 2 diabetic subjects. We studied 123 type 2 diabetic patients (67.5% male, aged 59.3+-10.1 years, mean HbA1c 7.4%). Clinical features and fasting total cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol (LDLc, using Friedewald's equation and an alternative formula), apolipoprotein B (apoB), lipoprotein (a) and LDL particle size (on gradient polyacrylamide gel electrophoresis) were assessed. Patients with phenotypes A (predominant LDL size gtoreq25.5 nm) and B (<25.5 nm) were compared, and regression analysis was performed to find the best markers of LDL particle. Cut-off points were obtained and evaluated as predictors of phenotype B (kappa index). Patients with phenotype B (36%) showed higher total cholesterol, triglyceride and apolipoprotein B, and lower HDL cholesterol and LDLc/apoB ratio. Triglyceride was the best predictor of LDL particle size ($r=-0.632$, $p<0.01$), but an LDLc/apoB ratio below 1.297 mmol/g detected phenotype B best (sensitivity 65.9%, specificity 92.4%, kappa=0.611). Although triglyceride concentration is the best predictor of LDL size in type 2 diabetes, LDLcholesterol/apolipoproteinB ratio is the best tool to detect phenotype B.

L7 ANSWER 62 OF 150 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 2002390868 EMBASE
TITLE: Short-term antioxidant supplementation reduces oxidative stress in elderly patients with type 2 diabetes mellitus - A pilot study.
AUTHOR: Nuttall S.L.; Martin U.; Kendall M.J.; Dunne F.
CORPORATE SOURCE: S.L. Nuttall, Queen Elizabeth Hospital, Edgbaston, Birmingham B15 2TH, United Kingdom
SOURCE: Practical Diabetes International, (2002) 19/7 (199-202).
Refs: 29
ISSN: 1357-8170 CODEN: PDINFY
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 003 Endocrinology
030 Pharmacology
037 Drug Literature Index
020 Gerontology and Geriatrics
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Aims: The aim of this pilot study was to determine what dose of which

antioxidants might be studied in clinical trials by assessing the impact of vitamin (C and E) supplementation on markers of oxidative stress and LDL subfractions in patients with type 2 diabetes mellitus. Methods: Nine elderly patients with type 2 diabetes took a moderate dose combination of vitamins C (500 mg) and E (400 IU) for 4 weeks. Following a 4 week washout, the patients had a further 4 weeks of supplementation with a higher dose combination of vitamins C (1000 mg) and E (800 IU). Blood was sampled pre- and post-supplementation for vitamin E by high-performance liquid chromatography (HPLC), total antioxidant capacity by enhanced chemiluminescence, total cholesterol and lipid hydroperoxides by colour spectrophotometry and LDL subfraction profile by disc polyacrylamide gel electrophoresis. Results: Vitamin E was increased, after the moderate dose combination (59.8 .+-. 6 versus 36.4 .+-. 4 .mu.mol/L, $p < 0.001$) and increased further by the higher dose (72.7 .+-. 11 versus 30.8 .+-. 5 .mu.mol/L, $p < 0.001$). Total antioxidant capacity was significantly increased above baseline after both doses (508.2 .+-. 33 versus 436.4 .+-. 31, $p < 0.01$ (moderate); 519.3 .+-. 48 versus 440.8 .+-. 34 .mu.mol/L trolox eq., $p < 0.01$ (high)). Lipid hydroperoxides were reduced more after the moderate dose combination than after the high dose (6.1 .+-. 1 versus 12.1 .+-. 2, $p < 0.01$; 8.0 .+-. 1 versus 11.6 .+-. 1 .mu.mol/L, $p < 0.05$). LDL subfraction score showed a non-significant reduction after both periods of supplementation. Conclusions: This study has demonstrated that supplementation with modest doses of the aantioxidant vitamins C and E can significantly increase antioxidant defences and reduce oxidative damage in elderly patients with type 2 diabetes.

L7 ANSWER 63 OF 150 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:488416 BIOSIS
DOCUMENT NUMBER: PREV200200488416
TITLE: Palmitate-induced apoptosis of microvascular endothelial cells and pericytes.
AUTHOR(S): Yamagishi, Sho-ichi (1); Okamoto, Tamami; Amano, Shinjiro; Inagaki, Yosuke; Koga, Kohachiro; Koga, Mari; Choei, Hiroshi; Sasaki, Nobuyuki; Kikuchi, Seiji; Takeuchi, Masayoshi; Makita, Zenji
CORPORATE SOURCE: (1) Division of Endocrinology and Metabolism, Department of Medicine, Kurume University School of Medicine, Kurume, 830-0011: shoichi@med.kurume-u.ac.jp Japan
SOURCE: Molecular Medicine (Baltimore), (April, 2002) Vol. 8, No. 4, pp. 179-184. print.
ISSN: 1076-1551.
DOCUMENT TYPE: Article

LANGUAGE: English

AB Background: Recent observations in the EURODIAB Complications Study demonstrated that markers of insulin resistance are strong risk factors for retinopathy incidence in patients with diabetes. However, the molecular mechanism underlying this remains to be elucidated. In this study, we investigated the influence of palmitate, a major saturated free fatty acid in plasma, on the apoptotic cell death of cultured microvascular endothelial cells (EC) and retinal pericytes. Materials and Methods: The intracellular formation of reactive oxygen species (ROS) was detected using the fluorescent probe CM-H2DCFDA. DNA synthesis was determined by measuring (3H)-thymidine incorporation into cells. DNA fragmentations of EC were quantitatively analyzed in an enzyme-linked immunosorbent assay, and DNA laddering was evaluated on agarose gel electrophoresis. Results: Palmitate increased ROS generation in microvascular EC. Furthermore, palmitate significantly inhibited DNA synthesis and induced apoptotic cell death in EC, which were completely prevented by an antioxidant, N-acetylcysteine. Palmitate up-regulated pericyte mRNA levels of a receptor for advanced glycation end products (AGE), and thereby potentiated the apoptotic effects of AGE on pericytes. Conclusions: The results suggest that palmitate could induce apoptotic cell death in microvascular EC and pericytes through the overgeneration of intracellular ROS, and thus be involved in the development of diabetic retinopathy.

L7 ANSWER 64 OF 150 MEDLINE

ACCESSION NUMBER: 2002057573 MEDLINE

DOCUMENT NUMBER: 21629649 PubMed ID: 11757011

TITLE: Synovial matrix metalloproteinase-2 in different stages of sheep temporomandibular joint osteoarthrosis.

AUTHOR: Miyamoto Ken; Ishimaru Jun-Ichi; Kurita Kenichi; Goss Alastair N

CORPORATE SOURCE: Department of Oral and Maxillofacial Surgery, Gifu University School of Medicine, Tsukasamachi, Gifu, Japan.. kenmiya@cc.gifu-u.ac.jp

SOURCE: JOURNAL OF ORAL AND MAXILLOFACIAL SURGERY, (2002 Jan) 60 (1) 66-72.
Journal code: 8206428. ISSN: 0278-2391.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Abridged Index Medicus Journals; Dental Journals; Priority Journals

ENTRY MONTH: 200201

ENTRY DATE: Entered STN: 20020125
Last Updated on STN: 20020128

Entered Medline: 20020125

AB PURPOSE: The purpose of this study was to test for the presence of matrix metalloproteinase (MMP) in synovial fluid of sheep with experimentally induced temporomandibular joint (TMJ) osteoarthrosis (OA) at various time intervals. MATERIALS AND METHODS: Twenty-one sheep were used in this study. TMJ OA was induced bilaterally in 18 sheep and 3 sheep were used as controls. Each experimental group had 3 sheep, and the groups were killed at 2, 4, 6, 8, 12, and 24 weeks postoperatively. Synovial fluid was collected at killing, and the joints were evaluated histologically. Gelatin zymography was performed to detect the presence of MMPs in the synovial fluid. RESULTS: TMJ OA gradually progressed over time. ProMMP-2 was detected in all experimental groups. However, the activated form of MMP-2 was only detected at 2 and 4 weeks postoperatively. CONCLUSION: Activated MMP-2 activity correlates with initial articular cartilage destruction rather than with the progression of OA in the sheep TMJ.

L7 ANSWER 65 OF 150 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 2003:142756 BIOSIS

DOCUMENT NUMBER: PREV200300142756

TITLE: Alpha-Fodrin is Cleaved by Caspase-3 in a Chronic Ocular Hypertensive (COH) Rat Model of Glaucoma.

AUTHOR(S): Tahzib, N. G. (1); Ransom, N. L. (1); Reitsamer, H. A.; McKinnon, S. J. (1)

CORPORATE SOURCE: (1) Department of Ophthalmology, University of Texas Health Science Center, San Antonio, TX, USA USA

SOURCE: ARVO Annual Meeting Abstract Search and Program Planner, (2002) Vol. 2002, pp. Abstract No. 986. cd-rom.
Meeting Info.: Annual Meeting of the Association For Research in Vision and Ophthalmology Fort Lauderdale, Florida, USA May 05-10, 2002

DOCUMENT TYPE: Conference

LANGUAGE: English

AB Purpose: Alpha-fodrin, a known caspase target, is a neuronal cytoskeleton protein. Its proteolysis is suggested to contribute to structural rearrangements including membrane blebbing during apoptosis. It is cleaved by caspase-3 during apoptosis and co-localizes with the formation of neurofibrillary tangles in Alzheimer's disease. We sought to determine whether alpha-fodrin is cleaved by caspase-3 in retinas of COH rats and whether this process is reduced by adeno-associated virus (AAV)-induced retinal ganglion cell (RGC) expression of X-linked inhibitor of apoptosis protein (XIAP), a potent

caspase-3 inhibitor. Methods: Ocular hypertension was induced unilaterally in eyes of brown Norway rats by limbal injection of hypertonic saline (2.0 M). Protein from COH and paired control retinas (n=5 each) were electrophoresed on a 10% Tris-HCl gel. Western immunoblotting was performed using a mouse monoclonal antibody to full-length alpha-fodrin. Densitometry of COH and control bands with subsequent paired t-tests were performed to determine significant differences in caspase-3 specific alpha-fodrin cleavage between COH and control retinas. In a similar experiment, COH was induced unilaterally in eyes pre-treated with an intravitreal injection of 2 mul of AAV-XIAP (n=4). Western immunoblots of retinal protein were performed in an identical fashion. Results: Caspase-3 cleavage of alpha-fodrin yields a specific protein fragment of 120 kDa. Densitometry of COH retina immunoblots showed significantly more cleavage of alpha-fodrin into 120 kDa fragments than control retinas ($p<0.01$, paired t-test). In addition, inhibition of retinal caspase-3 activity with XIAP gene therapy reduced the levels of the 120 kDa alpha-fodrin fragment compared to paired control retinas. Conclusion: Our results confirm our previous finding that caspase-3 is activated in the retina in a COH rat model of glaucoma, and that it cleaves alpha-fodrin, thereby contributing to the pathophysiology of glaucoma. This process parallels the pathology seen in other chronic neurodegenerations such as Alzheimer's disease, in which neurons undergo chronic caspase activation, slow build-up of cleavage products, and a delayed course of apoptosis. If caspase activation in glaucoma leads to protracted rather than rapid apoptosis of RGCs, a much longer therapeutic window exists for inhibition of apoptosis with caspase inhibitors such as XIAP.

L7 ANSWER 66 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-07515 BIOTECHDS
TITLE: Novel isolated human thioredoxin polypeptide, 22109 useful for treating pain, viral diseases, cellular proliferative and/or differentiative disorders and bone, immune, cardiovascular, liver and metabolic disorders; recombinant protein gene production, vector expression in host cell, sense, antisense and antibody useful in disease gene therapy
AUTHOR: BANDARU R
PATENT ASSIGNEE: MILLENNIUM PHARM INC
PATENT INFO: WO 2001096392 20 Dec 2001
APPLICATION INFO: WO 2000-US19544 15 Jun 2000
PRIORITY INFO: US 2000-211673 15 Jun 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-098054 [13]

AN 2002-07515 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - An isolated 22109 polypeptide (I) belonging to human thioredoxin family member, is new.

DETAILED DESCRIPTION - An isolated 22109 polypeptide (I) belonging to human thioredoxin family member, is new. (I) is selected from: (a) a fragment of a polypeptide comprising a 332 residue amino acid sequence (S1), fully defined in the specification, where the fragment comprises at least 15 contiguous amino acids of S1; (b) a naturally occurring allelic variant of a polypeptide comprising S1, where the polypeptide is encoded by a nucleic acid molecule which hybridizes to a 1946 or 999 base pair sequence (S2), fully defined in the specification, or its complement under stringent conditions; and (c) a polypeptide which is encoded by a nucleic acid at least 80 % identical to S2. INDEPENDENT CLAIMS are also included for the following: (1) an isolated nucleic acid molecule (II) selected from: (a) a nucleic acid molecule comprising at least 80 % identity to S2; (b) a nucleic acid molecule comprising a fragment of at least 450 nucleotides of S2; (c) a nucleic acid molecule which encodes a polypeptide comprising S1, or its fragment comprising 15 contiguous amino acids of S1; and (d) a nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide comprising S2, where the nucleic acid molecule hybridizes to a nucleic acid molecule comprising S2, or its complement under stringent conditions; (2) a host cell (III) containing (II); (3) an antibody (IV) which selectively binds (I); (4) producing (I), comprising culturing (III) under expression conditions, and recovering the polypeptide; (5) detecting (M1) the presence of (I) in a sample involves contacting the sample with a compound which selectively binds to (I), and determining if the compound binds to (I) in the sample; (6) detecting (M2) the presence of (II) in a sample, comprising: (a) contacting the sample with a nucleic acid probe or primer which selectively hybridizes to (II); and (b) determining if the nucleic acid probe or primer binds to (II) in the sample; (7) a kit (V) comprising a compound which selectively binds to (I) or hybridizes to (II), and instructions for use; (8) identifying (M3) a compound which binds to (I), comprising: (a) contacting (I), or a cell expressing (I) with a test compound; and (b) determining if (I) binds to the test compound; (9) modulating (M4) the activity of (I), comprising contacting (I) or a cell expressing (I) with a compound which binds to (I) in a sufficient concentration to modulate the activity of (I); and (10) identifying (M5) a compound which modulates the activity of (I), comprising: (a) contacting (I) with a test compound; and (b) determining the effect of the test compound on the activity of (I).

WIDER DISCLOSURE - Disclosed as new are the following: (1) a nucleic acid construct that includes (II); (2) a vector containing (II); (3) a nucleic acid fragment suitable as primer or hybridization probe for the detection of (II); (4) an isolated nucleic acid molecule

antisense to (II); (5) modulating expression or activity of (II); (6) evaluating the efficacy of a therapeutic or prophylactic agent; (7) an assay for determining the presence or absence of a genetic alteration in (I) or (II); (8) a nucleic acid molecule that differ from S2; (9) detectably labeled oligonucleotide primer and probe molecules; (10) 22109 chimeric or fusion proteins; (11) a fragment of (IV); (12) a nucleic acid molecule which encodes (IV); (13) a vector which includes the above mentioned nucleic acid; (14) a cell transformed with the above mentioned nucleic acid; (15) a cell line, e.g. hybridoma, for producing (V); (16) a non-human transgenic animal in which a 22109 gene has been introduced or disrupted; (17) a population of cells from the above mentioned transgenic animal; (18) an agent which modulates expression or activity of (I) or (II); (19) a kit for detecting the presence of 22109 in a biological sample; (20) a computer medium having a number of digitally encoded data records; (21) evaluating a sample or subject; (22) an array that includes a substrate having a number of addresses;(23) analyzing 22109, e.g. analyzing structure, function or relatedness to other nucleic acid or amino acid sequences; (24) making computer readable record of a 22109 sequence; (25) a machine-readable medium for holding instructions for performing a method for determining if a subject has a 22109-associated disease or disorder or a pre-disposition to the disease or disorder; and (26) determining if a subject has a 22109-associated disease or disorder or a pre-disposition to the disease or disorder, provided in an electronic system and/or in a network.

BIOTECHNOLOGY - Preferred Polypeptide: (I) further comprises heterologous amino acid sequences. Preferred Polynucleotide: (II) further comprises vector nucleic acid sequences and a nucleic acid sequence encoding a heterologous polypeptide. (II) comprises at least 80 % identical to a 1946 base pair sequence (S2), fully defined in the specification, a nucleic acid molecule comprising a Fragment of at least 700 nucleotides of S2, and a nucleic acid molecule which encodes a fragment of S1, where the fragment comprises at least 210 contiguous amino acids of S1. Preferred Cell: (III) is a mammalian host cell, preferably a non-human mammalian host cell. Preferred Method: In M1, the compound which binds to (I) is an antibody. In M2, the sample comprises mRNA molecules and is contacted with a nucleic acid probe.

ACTIVITY - Cytostatic; Osteopathic; Immunosuppressive; Antidiabetic; Antirheumatic; Antiarthritic; Neuroprotective; Antipsoriatic; Antiulcer; Antiasthmatic; Antiallergic; Hypotensive; Antiarteriosclerotic; Virucide; Antiinflammatory; Anorectic; Analgesic. No biological data is given.

MECHANISM OF ACTION - Gene therapy; modulator of cellular defense mechanisms against oxidative damage; regulator of glucocorticoid responsiveness by cellular oxidative stress response pathways; modulator of protein processing, protein folding and protein secretion; modulator of cardiovascular activities; regulator of a molecular chaperone.

USE - (I) or (II) is useful treating cellular proliferative and/or

differentiative disorders (e.g. cancer), bone disorders (e.g. osteoporosis, rickets), immune disorders (such as autoimmune diseases e.g. diabetes mellitus, rheumatoid arthritis, multiple sclerosis, psoriasis, Sjogren's Syndrome, Crohn's disease, ulcerative colitis, asthma, graft-versus-host disease, allergy), cardiovascular disorder (e.g. hypertension, atherosclerosis), liver disorders, viral diseases, pain, metabolic disorders (e.g. obesity), cellular stress-related disorders and hematopoietic stem cell related disorders. (I), (II) or (IV) is useful in screening assays, detection assays (e.g. chromosomal mapping, tissue typing, forensic biology), predictive medicine (e.g. diagnostic assays, prognostic assays, monitoring clinical trials and pharmacogenomics), and in methods of treatment (e.g. therapeutic and prophylactic). (I) or (IV) is useful as reagents or targets in assays applicable to treatment and diagnosis of 22109-mediated or -related disorders. (I) or (II) is useful as query sequences to perform a search against public databases to, for example identify other family members or related sequences. (I) is useful as an immunogen to generate antibodies that bind (I). (I) is useful to screen for naturally occurring 22109 substrates, and to screen for drugs or compounds which modulate 22109 activity. (I) is useful as a bait protein in a yeast two-hybrid or three-hybrid assay. (II) is useful as a hybridization probe to identify (II), or as polymerase chain reaction (PCR) primers for the amplification or mutation of (II). (II) is useful for gene therapy. (II) is useful to express (I), to detect 22109 mRNA or a genetic alteration in a 22109 gene, and to modulate 22109 activity. (IV) is useful to isolate and purify (I), to detect (I) and to diagnostically monitor protein levels in tissue as part of a clinical testing procedure.

ADMINISTRATION - A pharmaceutical composition comprising (I), (II) or (V) is administered by parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g. inhalation), transdermal (topical), transmucosal, or rectal route at a dose of 0.001-30, preferably 1-10, more preferably 5-6 mg/kg.

EXAMPLE - 22109 was expressed as a recombinant glutathione-S-transferase (GST) fusion polypeptide in Escherichia coli and the fusion polypeptide was isolated and characterized. Specifically, 22109 was fused to GST and this fusion polypeptide was expressed in E. coli, e.g. strain PEB199. Expression of the GST-22109 fusion protein in PEB199 was induced with isopropyl-B-D-thiogalactopyranoside (IPTG). The recombinant fusion polypeptide was purified from crude bacterial lysates of the induced PEB199 strain by affinity chromatography or glutathione beads. Using polyacrylamide gel electrophoresis analysis of the polypeptide purified from the bacterial lysates, the molecular weight of the resultant fusion polypeptide was determined.(123 pages)

L7 ANSWER 67 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-09242 BIOTECHDS
TITLE: New extracellular messenger polypeptides and polynucleotides encoding them, useful for diagnosing, treating or preventing e.g. neurological, autoimmune, inflammatory, developmental and endocrine disorders;
vector-mediated gene transfer, expression in host cell, transgenic animal and antibody for recombinant protein production, drug screening and disease gene therapy
AUTHOR: LAL P; YUE H; HE A; NGUYEN D B; WALIA N; GANDHI A R; AZIMZAI Y; BANDMAN O; TANG Y T; LU Y; BAUGHN M R; DUGGAN B M; LEE S; HAFALIA A; POLICKY J L
PATENT ASSIGNEE: INCYTE GENOMICS INC
PATENT INFO: WO 2001094587 13 Dec 2001
APPLICATION INFO: WO 2000-US18476 6 Jun 2000
PRIORITY INFO: US 2000-249019 14 Nov 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-154573 [20]
AN 2002-09242 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - A new isolated polypeptide (I) comprising: (a) a defined 304, 1438, 208, 159, 500, 455, 121, 55, or 545 amino acid sequence given in the specification; (b) a naturally occurring polypeptide comprising an amino acid sequence at least 90% identical to (a); or (c) a immunogenic or biologically active fragment of (a).
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated polynucleotide (II) encoding (I), or comprising: (a) a defined sequence of 1374, 4541, 1117, 2460, 2601, 2791, 709, 753 or 3413 bp (designated S1-S9, respectively) fully given in the specification; (b) a naturally occurring polynucleotide comprising a sequence at least 90% identical to (a); (c) a complement of (a) or (b); or (d) an RNA equivalent of (a)-(c); (2) a recombinant polynucleotide comprising a promoter sequence operably linked to (II); (3) a cell transformed with (II); (4) a transgenic organism comprising (II); (5) methods of producing, detecting or purifying (I); (6) an isolated antibody which specifically binds to (I); (7) an isolated polynucleotide comprising at least 60 contiguous nucleotides of the polynucleotide; (8) methods for detecting a target polynucleotide in a sample; (9) a composition comprising (I), an agonist or antagonist of (I), or an antibody of (6), and a pharmaceutical

excipient; (10) a method for treating a disease or condition associated with decreased expression or overexpression of functional extracellular messengers (XMES), by administering to a patient a composition of (10); (11) methods for screening a compound for effectiveness as an agonist or antagonist of (I); (13) (12) methods of screening for a compound that specifically binds to (I) or that modulates the activity of (I); (13) a method for screening a compound for effectiveness in altering expression of a target polynucleotide comprising (II); (14) a method for assessing toxicity of a test compound; (15) a diagnostic test for a condition or disease associated with the expression of XMES in a biological sample; (16) a method of diagnosing a condition or disease associated with the expression of XMES in a subject by administering a composition comprising the antibody of (6); (17) methods of preparing a polyclonal or monoclonal antibody that specifically binds to (I); and (18) an antibody produced by the method of (17), and a composition comprising the antibody and a suitable carrier.

BIOTECHNOLOGY - Preparation: (I) is produced by culturing a cell under conditions for the expression of the polypeptide, where the cell is transformed with a recombinant polynucleotide comprising a promoter sequence operably linked to a (I), and recovering the polypeptide so expressed. Preferred Methods: The method for detecting a target polynucleotide in a sample, comprises hybridizing the sample with a probe comprising at least 20 contiguous nucleotides of a sequence complementary to the target polynucleotide in the sample, and which specifically hybridizes to the target polynucleotide, under conditions where a hybridization complex is formed between the probe and the target polynucleotide or its fragments; and detecting the presence or absence of the hybridization complex, and, optionally, if present, the amount of the complex. The probe preferably comprises at least 60 contiguous nucleotides. The method may alternatively comprise amplifying the target polynucleotide or its fragment by PCR, and detecting the presence or absence of the amplified target polynucleotide or its fragment, and, optionally, if present, the amount of the amplified polynucleotide. A compound can be screened for effectiveness as an agonist of (I) by exposing a sample comprising (I) to a compound, and detecting the agonist activity in the sample. Screening for a compound that specifically binds to (I) comprises combining (I) with at least one test compound, and detecting binding of (I) to the test compound. Screening for a compound that modulates the activity of (I) comprises combining (I) with at least one test compound under conditions permissive for the activity of (I); assessing the activity of (I) in the presence of the test compound, and comparing the activity of (I) in the presence of the test compound with that in the absence of the test compound, where a change in the activity of (I) in the presence of the test compound is indicates that the compound is a modulator of the

activity of (I). Screening a compound for effectiveness in altering the expression of a target polynucleotide comprising (II) comprises exposing a sample containing the target polynucleotide to a compound under conditions suitable for the expression of the target polynucleotide, detecting altered expression of the target polynucleotide, and comparing the expression of the target polynucleotide in the presence of varying amounts of the compound and in the absence of the compound. The toxicity of a test compound can be assessed by: (a) treating a biological sample containing nucleic acids with the test compound; (b) hybridizing the nucleic acids of the treated biological sample with a probe comprising at least 20 contiguous nucleotides of (II) under conditions where a specific hybridization complex is formed between the probe and a target polynucleotide in the biological sample, (c) quantifying the amount of hybridization complex; and (d) comparing the amount of hybridization complex in the treated biological sample with the amount of hybridization complex in an untreated biological sample, where a difference in the amount of hybridization complex in the treated biological sample indicates toxicity of the test compound. A diagnostic test for a condition or disease associated with the expression of XMES in a biological sample comprises combining the biological sample with an antibody which specifically binds to (I), under conditions allowing antibody to bind to the polypeptide and form an antibody:polypeptide complex, and detecting the complex, the presence of which correlates with the presence of the polypeptide in the biological sample. Preferred Antibody: The antibody is selected from a chimeric antibody, a single chain antibody, a Fab fragment, a F(ab')2 fragment, and a humanized antibody. A polyclonal antibody having a specificity of the antibody that specifically binds to (I) can be prepared by: (a) immunizing an animal with (I) or its immunogenic fragment, under conditions to elicit an antibody response; (b) isolating antibodies from the animal; and (c) screening the isolated antibodies with the polypeptide. A monoclonal antibody having a specificity of the antibody that specifically binds to (I) can be prepared by: (a) immunizing an animal with a (I) or its immunogenic fragment, under conditions to elicit an antibody response; (b) isolating antibody producing cells from the animal; (c) fusing the antibody producing cells with immortalized cells to form a monoclonal antibody-producing hybridoma cells; (d) culturing the hybridoma cells; and (e) isolating the monoclonal antibody which binds specifically to (I). The antibody that specifically binds to (I) is produced by screening a Fab expression library or a recombinant immunoglobulin library. A method of detecting (I) comprises incubating an antibody that specifically binds to (I) with a sample under conditions allowing the specific binding of the antibody and the polypeptide; and detecting specific binding, which indicates the presence of (I) in the sample. Purifying (I) from a sample comprises incubating an antibody that

specifically binds to (I) with a sample under conditions allowing specific binding of the antibody and the polypeptide; and separating the antibody from the sample and obtaining the purified polypeptide.

ACTIVITY - Neuroprotective; nootropic; immunosuppressive; anti-inflammatory; cytostatic; antiviral; antibacterial; antifungal; parasitic; protozoal; antihelminthic; antidiabetic.

MECHANISM OF ACTION - Gene therapy.

USE - (I) may be used in screening for compounds that act as agonists/antagonists of (I), that specifically bind to (I), and in preparing polyclonal antibodies (claimed). The polypeptides and nucleic acids are useful in the diagnosis, treatment and prevention of neurological disorders (e.g. epilepsy, stroke, or Alzheimer's disease), autoimmune/inflammatory disorders (e.g. AIDS, Addison's disease, or allergies), developmental disorders (e.g. renal tubular acidosis, anemia or Cushing's syndrome), endocrine disorders (e.g. hypophysectomy, aneurysm or vascular malformation), and cell proliferative disorders (e.g. cancer), and in the assessment of the effects of exogenous compounds on the expression of nucleic acid and amino acid sequences of extracellular messengers. These may also be used in the treatment of viral, bacterial , fungal, parasitic, protozoal and helminthic infections, trauma, disorders associated with hypopituitarism, hypothyroidism, hyperthyroidism or gonadal steroid hormones, and pancreatic disorders such as type I or type II diabetes mellitus. The polynucleotides may be used for somatic or germline gene therapy.

ADMINISTRATION - Administration can be through oral, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, pulmonary, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual or rectal means. Dosage is 0.1-100000 mg, up to a total dose of about 1 g.

EXAMPLE - cDNA was synthesized and cDNA libraries were constructed with the UNIZAP vector system. Reverse transcription was initiated using oligo d(T) or random primers. Synthetic oligonucleotide adapters were ligated to double stranded cDNA, which was then digested with a restriction enzyme, size-selected using preparative agarose gel electrophoresis, and ligated into compatible restriction enzyme sites of the polylinker of a plasmid. Recombinant plasmids were transformed into competent E. coli cells. Plasmids obtained were recovered from host cells by in vivo excision, purified, and were resuspended in 0.1 ml of distilled water and stored. Incyte cDNA recovered from plasmids were sequenced. The polynucleotide sequences derived from Incyte DNAs were validated by removing vector, linker, and poly(A) sequences and by masking ambiguous bases, using algorithms and programs based on BLAST, dynamic programming, and dinucleotide nearest neighbor analysis. The Incyte cDNA sequences of translations were then queried against a selection of public databases such as the GenBank

primate, rodent, mammalian, vertebrate, and eukaryote databases, and BLOCKS, PRINTS, DOMO, PRODOM, and hidden Markov model (HMM)-based protein family databases such as PFAM. The full-length polynucleotide sequences were translated to derive the corresponding full-length polypeptide sequences. Full-length polypeptide sequences were subsequently analyzed by querying against databases such as the GenBank protein databases (genpept), SwissProt, BLOCKS, PRINTS, DOMO, PRODOM, Prosite, and hidden Markov model (HMM)-based protein family databases such as PFAM. (123 pages)

L7 ANSWER 68 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-10908 BIOTECHDS
TITLE: New antibody specific for human tumor necrosis factor (TNF)-alpha, useful for treating TNF-alpha-mediated diseases, e.g. congestive heart failure, septic or endotoxic shock, cachexia, adult respiratory distress syndrome; vector plasmid pTTO-mediated gene transfer and expression in Escherichia coli and Fab antibody for use in rheumatoid arthritis, osteoarthritis, cardiovascular disease, respiratory disease, AIDS, allergy, psoriasis,tuberculosis, inflammatory bone disorder, blood coagulation disorder, autoimmune disease, vulnerary and transplantation therapy
AUTHOR: ATHWAL D S; BROWN D T; WEIR A N C; POPPLEWELL A G; CHAPMAN A P; KING D J
PATENT ASSIGNEE: CELLTECH R and D LTD
PATENT INFO: WO 2001094585 13 Dec 2001
APPLICATION INFO: WO 2000-GB2477 6 Jun 2000
PRIORITY INFO: GB 2000-13810 6 Jun 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-216732 [27]
AN 2002-10908 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - An antibody molecule having specificity for human tumor necrosis factor-alpha (TNFalpha), comprising a heavy or light chain, is new.
DETAILED DESCRIPTION - An antibody molecule having specificity for human tumor necrosis factor-alpha (TNFalpha), comprising a heavy or light chain, is new. The variable domain of the heavy chain comprises a complementarily determining region (CDR) having the sequence given as H1 (AspTyrGlyMetAsn) for CDRH1, as H2' (TrpIleAsnThrTyrIleGlyGluProIleTyrAla AspSerValLysGly), or as H2' (TrpIleAsnThrTyrIleGlyGluProIleTyrValAspAspPh

eLysGly) for CDRH2, or as H3 (GlyTyrArgSerTyrAlaMetAspTyr) for CDRH3. The variable domain of the light chain comprises a CDR having the sequence given as L1 in (LysAlaSerGlnAsnValGlyThrAsnValAla) for CDRL1, as L2 (SerAlaSerPheLeuTyrSer) for CDRL2, or as L3 (GlnGlnTyrAsnIleTyrProLeuThr) for CDRL3. INDEPENDENT CLAIMS are also included for the following: (1) antibody molecules having specificity for human TNFalpha, having a light and heavy chain comprising a 214 or 229 residue amino acid sequence, respectively, both fully defined in the specification; (2) a variant of the antibody molecule of (1) having an improved affinity for TNFalpha; (3) compounds comprising the antibody molecule which: (a) is covalently attached to an amino acid at or towards the C-terminal end of its heavy chain, an effector or reporter molecule; (b) is attached to one of the cysteine residues at the C-terminal end of the heavy chain, a lysyl-maleimide group where each amino group of the lysyl residue has covalently linked to it a methoxypoly(ethyleneglycol) residue having a molecular weight of 20000 Da; or (c) has one or more synthetic or naturally occurring polymers attached to one of the cysteine residues at the C-terminal end of the heavy chain; (4) an antibody molecule comprising a hybrid CDR having a truncated donor CDR sequence, where the missing portion of the donor CDR is replaced by a different sequence and forms a functional CDR; (5) a DNA sequence which encodes the heavy and/or light chain of the antibody molecule; (6) cloning or expression vector containing the DNA sequence, where vector is pDNAbEng-G1 or pTTO(CDP870); (7) a host cell transformed with the vector of (6); (8) a process of producing the antibody molecule by culturing the host cell of (7) and isolating the antibody molecule; (9) a therapeutic or diagnostic composition comprising the antibody molecule or the compound comprising the antibody; (10) a polypeptide having the sequence (S1). (S1) is AspTyrGlyMetAsn, TrpIleAsnThrTyrIleGlyGluProIleTyrAlaAspSerValLysGly, GlyTyrArgSerTyrAlaMetAspTyr, LysAlaSerGlnAsnValGlyThrAsnValAla, SerAlaSerPheLeuTyrSer, GlnGlnTyrAsnIleTyrProLeuThr, or TrpIleAsnThrTyrIleGlyGluProIleTyrValAspAspPheLysGly.

BIOTECHNOLOGY - Preferred Antibody: The antibody comprises the sequence TrpIleAsnThrTyrIleGlyGluProIleTyrAlaAspSerValLysGly for CDRH2. The antibody is preferably a CDR-grafted antibody molecule. The variable domain comprises human acceptor framework regions and non-human donor CDRs, where the human acceptor framework regions of the variable domain of the heavy chain are based on a human group 1 consensus sequence, and comprises non-human donor residues at positions 28, 38, 46, 60, 67, 69 and 71. The human acceptor framework regions of the variable domain of the heavy chain are alternatively based on a human group 3 consensus sequence, and comprise non-human donor residues at positions 27, 28, 30, 48, 49, 69, 71, 73, 76 and 78. The light chain variable region hTNF40-gLI and the heavy chain variable region gh3hTNF40.4 have 321 and 354 base pair sequences, respectively, both fully defined in the specification. The antibody is a Fab fragment comprising a heavy chain having a 216

residue amino acid sequence, and a light chain having a 214 residue amino acid sequence, both fully defined in the specification. The antibody molecule may also be a modified Fab fragment having at the C-terminal end of its heavy chain, one or more amino acids to allow attachment of an effector or reporter molecule. The additional amino acids form a modified hinge region containing one or two cysteine residues to which the effector or reporter molecule may be attached. The modified Fab fragment comprises a heavy chain having a 229 residue amino acid sequence, and a light chain having a 214 residue amino acid sequence, both fully defined in the specification. The antibody is a murine anti-TNFalpha monoclonal antibody hTNF40, or a chimeric antibody molecule comprising the light and heavy chain variable domains of the monoclonal antibody defined above. The variant of the antibody defined above is obtained by an affinity maturation protocol. The missing part of the CDR sequence of a hybrid CDR, which has a truncated donor CDR sequence, is from the antibody from which the framework regions of the antibody molecule are derived. The missing part of the CDR sequence is derived from a germline antibody having consensus framework regions. The CDRH2 of the heavy chain is hybrid in the antibody molecule. The truncation of the donor CDR is 1-8 amino acids, preferably 4-6 amino acids, and is made at the C-terminus of the CDR. The antibody can be produced by culturing an Escherichia coli comprising an E. coli expression vector comprising the DNA sequence encoding the heavy or light chain of the antibody, and isolating the antibody molecule, where the antibody molecule is targeted to the periplasm. Preferred Compound: The compound comprises an effector molecule consisting of one or more polymers which are optionally substituted straight or branched chain polyalkylene, polyalkenylene or polyoxyalkylene polymer, or a branched or unbranched polysaccharide. The polymer is preferably a methoxypoly(ethyleneglycol). Preferred DNA: The DNA sequence encoding the light and heavy chain antibody comprises a 321 or 354 base pair sequence, fully defined in the specification. The DNA may also comprise a sequence selected from two sequences of 354 base pairs given in the specification. The DNA may alternatively comprise a 648, 642 or 687 base pair sequence, all fully defined in the specification. The DNA sequence may be comprised in an expression vector which is an E. coli expression vector, specifically pTTO(CDP870).

ACTIVITY - Antirheumatic; antiarthritic; osteopathic; cardiant; anti-HIV (human immunodeficiency virus); antibacterial; immunosuppressive; antiallergic; antipsoriatic; tuberculostatic; immunomodulator; anti-inflammatory; vulnerary. No biological data is given.

MECHANISM OF ACTION - TNF-Antagonist-Alpha.

USE - The antibody or the compound comprising the antibody is useful for treating or manufacturing a medicament for treating a pathology mediated by TNFalpha, such as rheumatoid- or osteo-arthritis (claimed).

TNFalpha-mediated diseases which can be treated by the antibody include sepsis, congestive heart failure, septic or endotoxic shock, cachexia, adult respiratory distress syndrome, acquired immunodeficiency syndrome (AIDS), allergies, psoriasis, tuberculosis, inflammatory bone disorders, blood coagulation disorders, burns, rejection episodes following organ or tissue transplant, Crohn's disease, and autoimmune diseases, such as thyroiditis. The antibodies may also be used to reduce the side effects associated with TNFalpha generation during neoplasty therapy, to eliminate or reduce shock-related symptoms associated with the treatment or prevention of graft rejection by use of an anti-lymphocyte antibody, for treating multi-organ failure, or in the diagnosis and imaging of disease states involving elevated levels of TNF alpha.

ADMINISTRATION - Administration can be oral, intravenous, intramuscular, intraarterial, intramedullary, intrathecal, intraventricular, transdermal, transcutaneous, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, intravaginal or rectal routes. Dosage is 0.01-50, preferably 15 mg/kg.

EXAMPLE - Total RNA was prepared from 3x10 to the power 7 hTNF40 hybridoma cells. cDNA sequences coding for the variable domains of hTNF40 heavy and light chains were synthesized using reverse transcriptase to produce single stranded cDNA copies of the mRNA present in the total RNA, followed by polymerase chain reaction (PCR) on the cDNAs with specific oligonucleotide primers. cDNA was synthesized in a 20 micro-l reaction volume containing 50 mM Tris-HCl, 74 mM KCl, 10 mM dithiothreitol, 3 mM MgCl2, 0.5 hexanucleotide primer, 2 mg hTNF40 RNA and 200 units of Moloney Murine Leukemia virus reverse transcriptase. After incubation at 42 degrees C for 60 minutes, reaction was terminated by heating at 95 degrees C for 5 minutes. cDNA aliquots were subjected to PCR. Reactions were incubated at 95 degrees C for 5 minutes, cycle through 94 degrees C for 1 minute, 55 degrees C for 1 minute, and 72 degrees C for 1 minute. After 30 cycles, aliquots of each reaction were analyzed by electrophoresis on agarose gel. Light chain reactions containing 5' primer mixes from light chain pools 1, 2 and 7 produced bands with sizes consistent with full length Vl fragments while fraction from heavy chain reaction pool 3 produced a fragment with a size expected of a Vh gene. DNA fragments produced in the light chain reaction pool 2 were digested with the enzymes BstBI and Sp1I, concentrated by ethanol precipitation, and electrophoresed on 1.4 % agarose gel. DNA bands in the rage of 400 base pair were recovered and cloned by ligated into vector pMR15.1 which has been restricted with BstBI and SplI. After ligation, mixtures were transformed into Escherichia coli LM 1035 and plasmids from resulting bacterial colonies screened for inserts by digestion with BstBI and Sp1I. DNA fragments produced in heavy chain reaction pool 3 were digested with HindIII and ApaI, and cloned into vector pMR14 that had been restricted with HindIII and ApaI. Representative plasmids containing inserts were analyzed by nucleotide

sequencing. The determined nucleotide sequence and predicted amino acid sequence of the variable domain of the heavy chain of antibody hTNF40 was 354 base pair and 118 amino acids, respectively. The determined nucleotide sequence and predicted amino acid sequence of the variable domain of the heavy chain of antibody hTNF40 was 324 base pairs and 108 amino acids, respectively.(119 pages)

L7 ANSWER 69 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-19666 BIOTECHDS
TITLE: New non-coding sequences isolated upstream of human insulin regulated substance-2 gene, useful as marker for predicting, diagnosing and treating metabolic diseases or disorders e.g. obesity and diabetes;
human gene expression profiling using DNA probe and DNA primer for disease diagnosis and therapy
AUTHOR: SMITH U
PATENT ASSIGNEE: METCON MEDICIN AB
PATENT INFO: WO 2001094410 13 Dec 2001
APPLICATION INFO: WO 2000-SE1308 8 Jun 2000
PRIORITY INFO: SE 2000-2189 9 Jun 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-566443 [60]
AN 2002-19666 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - An isolated, substantially purified nucleotide sequence (I) which is a non-coding sequence upstream of human insulin regulated substance (IRS-2) gene, comprising a fully defined sequence (S1) of 53, 51, 52, 50, 61, 44, 63, 54, 62, 46, 56 or 59 base pairs as given in the specification or its homolog, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a nucleotide sequence which is complementary to (I) or hybridizes under stringent conditions to (I); (2) a nucleotide sequence induced in differentiated mammalian cells by the addition of a thiazolidinedione such as pioglitazone, where the sequence is complementary to (I); (3) a substance (II) identified as having insulin regulating properties using (I); (4) a pharmaceutical composition comprising (II); (5) a veterinary preparation comprising (II); and (6) determining if a patient in need of treatment with an IRS has the predisposition to respond to the treatment, characterized in that the activation of IRS-2 is measured, by determining the amount or relative increase/decrease of the IRS-2 protein, or the corresponding mRNA when administering the IRS to a sample of cells taken

from the patient.

BIOTECHNOLOGY - Preferred Nucleic Acid: A nucleotide sequence which hybridizes to (I) is preferred. The homolog of (I) has 70-98% sequence homology to (S1) and is induced in differentiated mammalian cells, preferably adipocytes by the addition of a thiazolidinedione, such as pioglitazone.

ACTIVITY - Antidiabetic; Anorectic.

MECHANISM OF ACTION - Insulin regulator. No supporting data is given.

USE - (I) or its homolog is useful as a marker for insulin regulating action in an assay for evaluating or screening substances for insulin regulating properties in vitro, where adipocytes, hepatic cells, muscle tissue cells or pancreatic cells are used as model cells. (I), its homolog, IRS-2 transcript, sequence information derived from an IRS-2 transcript, or (II) is useful for manufacture of a medicament for treating diabetes and obesity, and as a veterinary preparation. (I) is also useful for diagnosis of IRS-2 related metabolic disorders, diabetes and/or differentiating between various types or stages of the disorders, and for determining if a patient in need of treatment with an insulin regulating substance has the predisposition to respond to the treatment, comprising measuring activation of IRS-2 by determining the amount or relative increase/decrease of the IRS-2 protein or corresponding mRNA, where (I) is used as a marker when administering IRS to a sample of cells such as blood, adipocyte, muscle or liver cells taken from the patient (all claimed).

ADMINISTRATION - No administration details given.

ADVANTAGE - By using sequences of (I) or their closely related homologs as reporters, for evaluation of IRS-2 activation, a more accurate prediction and diagnosis is made possible.

EXAMPLE - 3T3-L1 fibroblasts were grown and differentiated into adipocytes. To study the acute effect of insulin, the cells were serum-deprived for 3 hours before adding 100 nM insulin for 15 minutes. Cell lysates were made. Total cellular RNA was isolated from the cells with guanidinium thiocyanate. Northern blot analyses were performed on total cellular RNA with labeled cDNA probes made against beta-actin as housekeeping gene, mouse insulin regulated substance (IRS-1) and mouse IRS-2, rodent GLUT4 and PKB/Akt using a polymerase chain reaction (PCR) fragment against protein kinase B beta (PKB beta) in a common sequence for PKB alpha, PKB beta and PKB gamma. 5' sequence (CGAGAGGCCGCGACCCAACAC) and 3' sequence (AGGCGGCCGCACATCATCTCGTA) were used as PCR primers. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transformed and immunoblotted with appropriate antibodies against the specific proteins. Immunoprecipitations were performed as described in

Rondinone et al., 1997 and individual proteins were detected by blotting with horseradish peroxidase-linked secondary antibodies and using enhanced chemiluminescence. The 10 Kb genomic sequence immediately upstream of the open reading frame of the human IRS-2 gene was sequenced. The expression of several genes involved in insulin signaling and action were examined after 4-48 hours exposure to different concentrations of pioglitazone (pio). However, only the IRS-2 gene expression was consistently increased. The IRS-2 mRNA included a major 7.2 Kb band but a minor band at 8.2 Kb was also frequently seen. Insulin alone did not change IRS-2 expression while the addition of pio increased mRNA 4-fold. This was due to an effect of pio alone and no further increase was seen by the addition of insulin. After 48 hours, the IRS-2 mRNA levels were consistently increased 3-5 fold relative to beta-actin mRNA. IRS-2 protein expression was also increased by pioglitazone but not changed by insulin. The average increase seen after 48 hours with pio was 36%. (38 pages)

L7 ANSWER 70 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-05433 BIOTECHDS
TITLE: Screening assays for identifying compounds useful for treating immune disorders, comprises identification of compounds that modulate alpha 2-macroglobulin receptor-heat shock protein interaction;
alpha-2-macroglobulin receptor protein, heat shock protein interaction useful for drug screening and disease therapy
AUTHOR: SRIVASTAVA P K
PATENT ASSIGNEE: UNIV CONNECTICUT HEALTH CENT
PATENT INFO: WO 2001092474 6 Dec 2001
APPLICATION INFO: WO 2000-US18041 2 Jun 2000
PRIORITY INFO: US 2000-750972 28 Dec 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-122061 [16]
AN 2002-05433 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Screening assays (M1) comprising identification of compounds that modulate alpha2-macroglobulin (alpha2M) receptor (which also functions as heat shock protein (HSP) receptor)-HSP interaction, is new.
DETAILED DESCRIPTION - M1 comprises: (a) identifying (I) a compound that modulates an HSP-alpha2M receptor-mediated process, by contacting a test compound with HSP and alpha2M receptor or alpha2M receptor-expressing cell, and measuring the level of alpha2M receptor activity or expression, such that if the level of activity or expression

measured in the presence of the compound differs from the level of alpha2M receptor activity in the absence of the test compound, then a compound that modulates an HSP-alpha2M receptor-mediated process is identified; (b) identifying (II) a compound that modulates the binding of HSP to alpha2M receptor, by contacting HSP with alpha2M receptor, its fragment, analog, derivative or mimetic, in the present of a test compound and measuring the amount of HSP bound to alpha2M receptor, its fragment, analog, derivative or mimetic, such that if the amount of bound HSP measured in the presence of the test compound differs from the amount of bound HSP measured in the absence of the test compound, then a compound that modulates the binding of an HSP to the alpha2M receptor is identified; (c) identifying (III) a compound that modulates HSP-mediated antigen presentation by alpha2M receptor-expressing cells, by adding a test compound to a mixture of alpha2M receptor expressing cells and a complex consisting essentially of HSP non-covalently associated with an antigenic molecule, under conditions conducive to alpha2M receptor-mediated endocytosis, measuring the level of stimulation of antigen-specific cytotoxic T cells by alpha2M receptor-expressing cells, such that if the level measured in the presence of the test compound differs from the level of the stimulation in the absence of the test compound, then a compound that modulates HSP-mediated antigen presentation by alpha2M receptor-expressing cells is identified;or (d) detecting (IV) a HSP-alpha2M receptor-related disorder in a mammal, by measuring the level of activity from an HSP-alpha2M receptor-mediated process in a patient sample, such that if the measured level differs from the level found in clinically normal individuals, then a HSP-alpha2M receptor-related disorder is detected. INDEPENDENT CLAIMS are also included for the following: (1) modulating (M2) an immune response, by administering to a mammal a purified compound that modulates the interaction of HSP with alpha2M receptor; (2) treating (M3) an autoimmune disorder, by administering to a mammal in need of such treatment a purified compound that interferes with the interaction of HSP with the alpha2M receptor; (3) treating an autoimmune disorder, by administering to a mammal in need of such treatment, a recombinant cell that expresses an alpha2M receptor which decreases the uptake of HSP by a functional alpha2M receptor; (4) increasing the immunopotency of a cancer cell or an infected cell; (5) increasing the immunopotency of a cancer cell or an infected cell, by transforming the cell with a nucleic acid comprising a nucleotide sequence that is operably linked to a promoter, and encodes an alpha2M receptor polypeptide, and administering the cell to individual in need of treatment, so as to obtain an elevated immune response; (6) a recombinant cancer cell or recombinant infected cell (V) transformed with (N); (7) a kit (K1); (8) a kit (K2), in one or more containers; (9) identifying an alpha2M receptor fragment capable of binding HSP, by contacting HSP or its peptide-binding fragment with one or more alpha2M receptor fragments, and identifying an alpha2M receptor

fragment which specifically binds to HSP or its peptide-binding fragment; (10) identifying (M4) an alpha2M receptor fragment capable of inducing an HSP-alpha2M receptor-mediated process, by contacting HSP with a cell expressing alpha2M receptor fragment and measuring the level of alpha2M receptor activity in the cell, such that if the level of HSP-alpha2M receptor-mediated process or activity measured is greater than the level of alpha2M receptor activity in the absence of the alpha2M receptor fragment, then an alpha2M receptor fragment capable of inducing an HSP-alpha2M receptor-mediated process is identified; (11) identifying HSP fragment capable of binding an alpha2M receptor, by contacting an alpha2M receptor with one or more HSP fragments and identifying HSP fragment which specifically binds to the alpha2M receptor; (12) identifying (M5) HSP fragment capable of inducing an HSP-alpha2M receptor-mediated process; (13) identifying (M6) a molecule that binds specifically to an alpha2M receptor; (14) screening for molecules that specifically bind to an alpha2M receptor; (15) identifying a compound that modulates the binding of an alpha2M receptor ligand to the alpha2M; (16) identifying a compound that modulates the interaction between the alpha2M receptor and an alpha2M receptor ligand; (17) identifying (M7) a compound that modulates antigen presentation by alpha2M receptor-expressing cells; (18) modulating an immune response, by administering to a mammal a purified compound that binds to the alpha2M receptor; (19) treating or preventing a disease or disorder, by administering to a mammal a purified compound that binds to the alpha2M receptor; (20) treating an autoimmune disorder, by administering to a mammal in need of such treatment a purified compound that binds to the alpha2M receptor; (21) stimulating (M8) an immune response in a patient, by administering to the patient blood which has been withdrawn from the patient and treated to remove an alpha2M receptor ligand; (22) stimulating (M9) an immune response in a patient, by removing alpha2M receptor ligand from blood withdrawn from the patient, and returning at least a portion of the alpha2M receptor ligand-depleted blood to the patient; (23) stimulating (M10) an immune response in a patient, by withdrawing blood from the patient, removing alpha2M receptor ligand from the blood and returning at least a portion of alpha2M receptor ligand-depleted blood to the patient; and (24) a kit (K3);

BIOTECHNOLOGY - Preffered Kit: K3 comprises in one or more containers, a solid phase chromatography column with a purified alpha2M receptor ligand binding molecule attached to it, such that withdrawn blood can be run over the column to deplete the blood of alpha2M receptor ligand. (K1) comprises in one or more containers an anti-alpha2M receptor antibody or a nucleic acid probe capable of hybridizing to an alpha2M receptor nucleic acid, a purified HSP, a nucleic acid encoding HSP, or cell expressing HSP and instructions for use in detecting HSP-alpha2M receptor-related disorder. (K2), comprises a purified HSP, nucleic acid encoding HSP, or cell expressing HSP and an alpha2M receptor polypeptide,

nucleic acid encoding an alpha2M receptor polypeptide, or cell expressing an alpha2M receptor polypeptide. Preferred Compound: The compound identified by M1 is an antagonist which interferes with the interaction of HSP with the alpha2M receptor. The test compound is an antibody specific for alpha2M receptor or HSP, a small molecule or a peptide. The peptide comprises at least 5 consecutive amino acids of alpha2M receptor (a sequence comprising 126 amino acids fully defined in the specification) or alpha2M (a sequence comprising 1474 amino acids fully defined in the specification). The peptide comprises at least 5 consecutive amino acids of HSP sequence. The compound is an agonist which enhances the interaction of HSP with alpha2M receptor. HSP is gp96, hsp90, hsp70 or calreticulin. The alpha2M receptor ligand is alpha2M. alpha2M receptor is on a cell surface or immobilized to a solid surface. The alpha2M receptor ligand-binding molecule is alpha2M receptor or its fragment, and it does not bind HSP. Alternately, the alpha2M receptor ligand-binding molecule is an alpha2M receptor ligand-specific antibody or its fragment. The alpha2M receptor ligand is alpha2M, a lipoprotein complex, lactoferrin, tissue-type plasminogen activator, urokinase-type plasminogen activator or exotoxin. Preferred Method: The method further involves determining whether the level interferes with the interaction of HSP and the alpha2M receptor. alpha2M receptor activity measured is the ability to interact with HSP. HSP is non-covalently associated with an antigenic peptide and the alpha2M receptor activity measured is the ability to represent the antigenic peptide or to stimulate a cytotoxic T-cell response against the antigenic peptide. In (I), the solid surface is a microtiter dish. The amount of bound HSP is measured by contacting the cell with HSP-specific antibody, or HSP is labeled with a fluorescent label, and the amount of bound HSP is measured by detecting the label. (IV) further involves contacting the sample derived from a patient with an antibody specific for alpha2M receptor, HSP or HSP-alpha2M complex under conditions such that immunospecific binding by the antibody. In M2, the compound is an agonist which enhances the interaction of HSP and alpha2M receptor, or an antagonist that interferes with the interaction between HSP and alpha2M receptor. The antagonist is a small molecule, peptide comprising at least 5 consecutive amino acids of alpha2M receptor, alpha2M or HSP, and is an antibody specific for alpha2M receptor or HSP. In M5, alpha2M receptor activity measured is the ability to interact with HSP. In M6, the test molecules are potential immunotherapeutic drugs. M7 further involves measuring stimulation of antigen specific cytotoxic T cells by the alpha2M receptor-expressing cells which involves adding the alpha2M receptor-expressing cells formed to T cells under conditions conducive to the activation of the T cells, and comparing the level of activation of said cytotoxic T cells with the level of activation of T cells by an alpha2M receptor-expressing cell formed in the absence of the test compound, where an increase or decrease in the level of T cell activation indicates that a compound that

modulates HSP-mediated antigen presentation by alpha2M receptor-expressing cells is identified. M8 further involves administering HSP or HSP-antigenic peptide complex to the patient. M10 further involves adding HSP or HSP-antigenic peptide complex to the blood, after withdrawing blood from the patient. M9 or M10 further involves removing alpha2M receptor ligand from the blood, by contacting the blood with a solid phase attached to a alpha2M receptor ligand-binding molecule for a time period and under conditions sufficient to allow binding of alpha2M receptor ligand to the alpha2M receptor ligand-binding molecule solid phase. An apheresis system is used for removing alpha2M receptor ligand from the blood which is withdrawn manually. The removal step involves separating the plasma from the blood and treating the plasma to remove the alpha2M receptor ligand. The blood is then administered to the patient by syringe or by an intravenous drip. Preferred Cell: (V) is a human cell. Preferred Kit: The antibody or nucleic acid probe in K1 is labeled with a detectable marker.
A1 further comprises a labeled macroglobulin receptor polypeptide. In K2, the alpha2M receptor polypeptide, the nucleic acid encoding the polypeptide or the cell expressing the polypeptide is purified. K2 further comprises instructions for use in treating an autoimmune disorder, infectious disease or proliferative disorder. (17) comprises adding one or more test compounds to a mixture of alpha2M receptor-expressing cells and a complex comprising an alpha2M receptor ligand and an antigenic molecule, under conditions conducive to alpha2M receptor-mediated endocytosis, measuring the level of stimulation of antigen-specific cytotoxic T cells by the alpha2M receptor-expressing cells, such that if the level measured differs from the level of said stimulation in the absence of the one or more test compounds, then a compound that modulates antigen presentation by alpha2M receptor-expressing cells is identified. (16) comprises contacting an alpha2M receptor with one or more test compounds and measuring the level of alpha2M receptor activity or expression, such that if the level of activity or expression measured differs from the level of alpha2M receptor activity in the absence of one or more test compounds, then a compound that modulates the interaction between the alpha2M receptor ligand is identified. (15) comprises contacting an alpha2M receptor with an alpha2M receptor ligand or an alpha2M receptor binding fragment, analog, derivative or mimetic, in the presence of one or more test compounds and measuring the amount of alpha2M receptor ligand, or fragment, analog, derivative or mimetic, bound to the alpha2M receptor, such that if the amount of bound alpha2M receptor ligand measured differs from the amount of bound alpha2M receptor measured in the absence of the test compound, then a compound that modulates the binding of an alpha2M receptor ligand to the alpha2M receptor is identified. (14) comprises contacting an alpha2M receptor comprising, contacting an alpha2M receptor with one or more test molecules under conditions conducive to binding,

and determining whether any of the test molecules specifically bind to the alpha2M receptor. (M5) comprises by contacting an alpha2M receptor fragment with a cell expressing HSP and measuring the level of alpha2M receptor activity in the cell, such that if the level of the HSP-approximately2M receptor-mediated process or activity measured is greater than the level of alpha2M receptor activity in the absence of HSP fragment, then HSP fragment capable of inducing an HSP-alpha2M receptor-mediated process is identified. (4) comprises transforming the cell with a nucleic acid (N) comprising a nucleotide sequence that is operably linked to a promoter, and encodes an alpha2M receptor polypeptide. (13) comprises contacting an alpha2M receptor with one or more test molecules under conditions conducive to binding and identifying one or more test molecules that specifically bind to the alpha2M receptor.

ACTIVITY - Immunosuppressive; antiinflammatory; cytostatic; virucide; antilipemic; nootropic; antidiabetic; osteopathic.

MECHANISM OF ACTION - Modulator of interaction between alpha2M receptor and HSP (claimed). No supporting data given.

USE - The interaction between alpha2M receptor and HSP is useful in screening assays for identifying compounds that modulate the interaction of alpha2M receptor and HSP. The identified compounds are useful for treating an autoimmune disorder, disease or disorder involving disruption of antigen presentation or endocytosis or cytokine clearance or inflammation, proliferative disorder, viral disorder or other infectious diseases, hypercholesterolemia, Alzheimer's disease, diabetes or osteoporosis (claimed).

ADMINISTRATION - The compounds identified by M1 are administered through standard administration routes. Dosage details not given.

EXAMPLE - gp96 molecules were coupled to the radio-iodinated linker sulfosuccinimidyl (4-azidosalicylamido)hexanoate (SASD) which contains a photo cross-linkable group. Gp960SASD-I125 was pulsed onto peritoneal macrophages, which have been shown previously to represent gp96-chaperoned peptides. Excess gp 96-SASD was removed by multiple rounds of washing of the cells and photoactivation was carried out by the exposure of cells to UV light for 10 minute. Cell lysates were reduced in order to transfer the I125 group to the putative gp96 ligand and were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by autoradiography. The gp96 molecule was observed to cross-link approximately 80 kDa band specifically present in representation-competent macrophage but not in the representation-incompetent P815 cells. The band appeared to correspond in size to the larger of the two bands seen in eluates of gp96 affinity columns. No band corresponding to the lower band in that preparation was seen in the photo cross-linked preparation. The observation of a specific binding of gp96 to an 80 kDa protein in two different representation-competent cell types, but not in a

representation-incompetent cell line, and by two independent assays supported the candidacy of the 80 kDa molecule for the gp96 receptor. (236 pages)

L7 ANSWER 71 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-09232 BIOTECHDS
TITLE: New human ion channel polypeptides and nucleic acids, useful for treating or diagnosing neurological, psychiatric or neurodegenerative diseases, e.g. depression, anxiety, stroke, ischemia, or Alzheimer's or Parkinson's disease; antibody, drug screening, and antisense DNA useful for gene therapy and diagnosis
AUTHOR: BENJAMIN C W; ROBERDS S L; KARNOVSKY A M; RUBLE C L; GOTOW L F
PATENT ASSIGNEE: PHARMACIA and UPJOHN CO
PATENT INFO: WO 2001092303 6 Dec 2001
APPLICATION INFO: WO 2000-US16967 26 May 2000
PRIORITY INFO: US 2000-207257 26 May 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-147617 [19]
AN 2002-09232 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - An isolated nucleic acid molecule (I), which encodes at least a portion of ion-x, is new.

DETAILED DESCRIPTION - (I) comprises a nucleotide sequence that encodes a polypeptide having a sequence homologous to a sequence of the human ion channels. (I) comprises a nucleotide sequence having 623, 573, 201, 744, 445, 701, 562, 596, 640, 691, 632, 553, 188, 560, 625, 679, 598, 457 or 550 bp (designated Ia-Is, respectively) fully defined in the specification. INDEPENDENT CLAIMS are also included for the following: (1) expression vectors comprising the nucleic acid molecule; (2) host cells transformed or transfected with the expression vector or polynucleotide; (3) isolated nucleic acid molecules comprising at least 10 nucleotides, which comprises a nucleotide sequence complementary to the sequence Ia-Is; (4) methods of producing the polypeptide; (5) isolated polypeptides encoded by (I); (6) isolated antibodies, which bind to an epitope on a polypeptide; (7) inducing an immune response in a mammal against the polypeptide by administering the polypeptide to induce the immune response; (8) identifying a compound that binds ion-x or an ion channel encoded by a sequence having Ic-Is; (9) identifying a compound that binds a nucleic acid molecule encoding ion-x or a compound that binds a nucleic acid molecule comprising Ic-Is; (10) identifying a compound that modulates the (biological) activity of ion-x or an ion

channel encoded by a sequence comprising Ic-Is; (11) compounds identified by the methods of (8), (9), (10) or (11); (12) identifying an animal homolog of ion-x; (13) screening a human subject to diagnose a disorder affecting the brain or a genetic predisposition for it, or for screening for an ion-x mental disorder genotype; (14) kits for screening a human subject to diagnose a mental disorder or a genetic predisposition for it; (15) identifying an ion channel allelic variant that correlates with a mental disorder; (16) identify compounds useful for the treatment of a disorder; (17) identifying a compound useful as a modulator of binding between ion-x or an ion channel, and a binding partner of ion-x or an ion channel; (18) chimeric receptors comprising at least 5 amino acid residues, the receptor comprising at least a portion of any of AA1-AA19; and (19) compositions comprising the nucleic acid molecule, the recombinant expression vector, polypeptide or antibody, and an acceptable carrier or diluent.

BIOTECHNOLOGY - Preferred Nucleic Acid: The isolated nucleic acid molecule comprises a sequence that encodes a polypeptide comprising a sequence 36, 46, 22, 29, 18, 40, 18, 25, 18, 31, 18, 28, 27, 22, 21, 51, 68, 44 or 55 amino acids (designated AA1-AA19) fully defined in the specification. The isolated nucleic acid molecule comprises any of the DNA sequences cited above or a sequence homologous to any of the DNA sequences cited above. The isolated nucleic acid molecule may be a DNA or RNA. The nucleic acid molecule of (3) is an antisense oligonucleotide directed to a region of a sequence selected from Ia-Is. The oligonucleotide is directed to a regulatory region of a sequence selected from Ia-Is. The purified and isolated polynucleotide comprises a nucleotide sequence encoding ion-x allelic variant. The purified polynucleotide comprises a nucleotide sequence encoding ion-x of a human with a mental disorder, where the polynucleotide hybridizes to the complement of Ia-Is under the following hybridization conditions: (a) hybridization for 16 hours at 42degreesC in a hybridization solution comprising 50% formamide, 1% SDS, 1 M NaCl, 10% dextran sulfate; and (b) washing 2 times for 30 minutes at 60degreesC in a wash solution comprising 0.1x SSC and 1% SDS, where the polynucleotide that encodes ion-x amino acid sequence of the human differs from the sequence selected from Ia-Is by at least one residue. Preferred Expression Vector: The expression vector is a plasmid or a viral particle. Preferably, the expression vector consists of adenoviruses, baculoviruses, parvoviruses, herpesviruses, poxviruses, adeno-associated viruses, Semliki Forest viruses, vaccinia viruses or retroviruses. The nucleic acid molecule is operably connected to a promoter selected from simian virus 40, mouse mammary tumor virus, long terminal repeat of human immunodeficiency virus, maloney virus, cytomegalovirus immediate early promoter, Epstein Barr virus, rous sarcoma virus, human actin, human myosin, human hemoglobin, human muscle creatine and human metalothionein. Preferred Host Cell: The transformed host cell is a bacterial cell (e.g.

Escherichia coli), yeast (e.g. Saccharomyces cerevisiae), insect cell (e.g. S. frugiperda) or mammalian cell (e.g. chinese hamster ovary cells, HeLa cells, African green monkey kidney cells, human HEK-293 cells or murine 3T3 fibroblasts). Preferred Method: In method (4), preparing the polypeptide comprises: (a) introducing the recombinant expression vector into a compatible host cell; (b) growing the host cell under conditions for expression of the polypeptide; and (c) recovering the polypeptide. The host cell is lysed and the polypeptide is recovered from the lysate of the host cell. The polypeptide is recovered by purifying the culture medium without lysing the host cell. In method (9), identifying a compound, which binds ion-x, comprises: (a) contacting ion-x with a compound; and (b) determining whether the compound binds ion-x. The ion-x comprises an amino acid sequence comprising AA1-AA19. The binding of the compound to ion-x is determined by a protein binding assay. In particular, the protein binding assay consists of a gel-shift assay, western blot, radiolabeled competition assay, phagebased expression cloning, co-fractionation by chromatography, co-precipitation, cross linking, interaction trap/two-hybrid analysis, southwestern analysis and ELISA. Identifying a compound that binds an ion channel encoded by a sequence comprising Ic-Is comprises: (a) contacting the ion channel with a compound; and (b) determining whether the compound binds the ion channel. The ion channel comprises an amino acid sequence comprising AA3-AA19. In method (9), identifying a compound that binds a nucleic acid molecule encoding ion-x comprises: (a) contacting the nucleic acid molecule encoding ion-x with a compound; and (b) determining whether the compound binds the nucleic acid molecule.The binding is determined by a gel-shift assay. Identifying a compound that binds a nucleic acid molecule having comprising Ic-Is may comprise: (a) contacting the nucleic acid molecule with a compound; and (b) determining whether the compound binds the nucleic acid molecule.In method (10), identifying a compound, which modulates the activity of ion-x comprises: (a) contacting ion-x with a compound; and (b) determining whether ion-x activity has been modulated. In particular, he activity is neuropeptide binding or neuropeptide signaling. Identifying a compound that modulates the activity of an ion channel may comprise: (a) contacting the ion channel with a compound; and (b) determining whether ion channel activity has been modulated. Identifying a modulator of biological activity of an ion channel encoded by a sequence selected from Ic and Is: (a) contacting a cell in the presence and in the absence of a putative modulator compound; (b) measuring ion channel biological activity in the cell. A decreased or increased ion channel biological activity in the presence versus absence of the putative modulator is indicative of a modulator of biological activity. In method (12), identifying an animal homolog of ion-x comprises: (a) comparing the nucleic acid sequences of the animal with Ia-Is; and (b) identifying nucleic acid sequences of the animal that are homologous to the sequence selected from Ia-Is. Comparing the nucleic

acid sequences of the animal with any of Ia-Is is performed by DNA hybridization or by computer homology search. In method (13), screening a human subject to diagnose a disorder affecting the brain or genetic predisposition for it comprises: (a) assaying nucleic acid of a human subject to determine a presence or an absence of a mutation altering an amino acid sequence, expression or biological activity of at least one ion channel that is expressed in the brain, where the ion channel comprises an amino acid sequence comprising AA1-AA19 or their allelic variants, and where the nucleic acid corresponds to a gene encoding the ion channel; and (b) diagnosing the disorder or predisposition from the presence or absence of the mutation, where the presence of a mutation altering the amino acid sequence, expression or biological activity of the ion channel correlates with an increased risk of developing the disorder. The assaying step comprises at least one procedure consisting of: (a) comparing nucleotide sequences from the human subject and reference sequences, and determining a difference of at least a nucleotide of at least one codon between the nucleotide sequences from the human subject that encodes an ion-x allele and an ion-x reference sequence;(b) performing a hybridization assay to determine whether nucleic acid from the human subject has a nucleotide sequence identical to or different from one or more reference sequences; (c) performing a polynucleotide migration assay to determine whether nucleic acid from the human subject has a nucleotide sequence identical to or different from one or more reference sequences; and (d) performing a restriction endonuclease digestion to determine whether nucleic acid from the human subject has a nucleotide sequence identical to or different from one or more reference sequences. Screening for an ion-x mental disorder genotype in a human patient comprises: (a) providing a biological sample comprising nucleic acid from the patient, the nucleic acid including sequences corresponding to alleles of ion-x; and (b) detecting the presence of one or more mutations in the ion-x alleles, where the presence of a mutation in an ion-x allele is indicative of a mental disorder genotype. In particular, the biological sample is a cell sample. Screening a human subject to diagnose a disorder affecting the brain or genetic predisposition for it may comprise: (a) assaying nucleic acid of a human subject to determine a presence or an absence of a mutation altering an amino acid sequence, expression or biological activity of at least one ion channel that is expressed in the brain, where the ion channel comprises AA3-AA19 and their allelic variants, and where the nucleic acid corresponds to a gene encoding the ion channel; and (b) diagnosing the disorder or predisposition from the presence or absence of the mutation, where the presence of a mutation altering the amino acid sequence, expression or biological activity of the ion channel correlates with an increased risk of developing the disorder. In method (15), identifying an ion channel allelic variant that correlates with a mental disorder comprises: (a) providing a biological sample comprising nucleic

acid from a human patient diagnosed with a mental disorder, or from the patient's genetic progenitors or progeny; and (b) detecting in the nucleic acid the presence of one or more mutations in an ion channel that is expressed in the brain, where the ion channel comprises an amino acid sequence selected from AA1-AA19 and their allelic variants, and where the nucleic acid includes sequence corresponding to the gene or genes encoding the ion channel. One or more mutations detected indicate an allelic variant that correlates with a mental disorder. In method (16), identifying compounds useful for the treatment of a disorder comprises: (a) contacting a composition comprising ion-x with a compound suspected of binding ion-x; and (b) detecting binding between ion-x and the compound suspected of binding ion-x; where compounds identified as binding ion-x are candidate compounds useful for the treatment of a disorder. The method may also comprise: (a) contacting a composition comprising the ion channel with a compound suspected of binding the ion channel; and (b) detecting binding between the ion channel and the compound suspected of binding the ion channel; where compounds identified as binding the ion channel are candidate compounds useful for the treatment of a disorder. In method (17), identifying a compound useful as a modulator of binding between ion-x or an ion channel, and a binding partner of ion-x or an ion channel comprises: (a) contacting the binding partner and a composition comprising ion-x or an ion channel in the presence and in the absence of a putative modulator compound; and (b) detecting binding between the binding partner, and ion-x or an ion channel; where decreased or increased binding between the binding partner and ion-x in the presence of the putative modulator, as compared to binding in the absence of the putative modulator is indicative a modulator compound useful for the treatment of a disorder. The composition comprises a cell expressing the ion channel on its surface. In particular, the composition comprises a cell transformed or transfected with a polynucleotide that encodes the ion channel or ion-x. Preferred Polypeptide: The polypeptide comprises a sequence selected from (AA1-AA19). The polypeptide comprises an amino acid sequence homologous to AA1-AA19. The sequence homologous to AA1-AA19 comprises at least one conservative amino acid substitution compared to AA1-AA19. The polypeptide comprises an allelic variant of a polypeptide with a sequence selected from AA1-AA19. Preferred Antibody: The antibody is a monoclonal antibody. Preferred Kit: The kit comprises, in association: (a) an oligonucleotide useful as a probe for identifying polymorphisms in a human ion-x gene, the oligonucleotide comprising 6-50 nucleotides in a sequence that is identical or complementary to a sequence of a wild type human ion-x coding sequence, except for one sequence difference selected from a nucleotide addition, a nucleotide deletion or nucleotide substitution; and (b) a media packaged with the oligonucleotide, the media containing information for identifying polymorphisms that correlate with a mental disorder or a genetic predisposition for it, the

polymorphisms being identifiable using the oligonucleotide as a probe.

ACTIVITY - Neuroleptic; nootropic; neuroprotective. No details of clinical tests given.

MECHANISM OF ACTION - Gene therapy; neurotransmitter-gated ion channel modulator.

USE - The nucleic acids, polypeptides and their modulators are useful for the treatment of human diseases and conditions such as neurological or psychiatric disorders. Ion channels are also useful targets for discovering ligands or drugs to treat many diverse disorders and defects. In particular, these compounds are useful for treating schizophrenia, depression, anxiety, attention deficit hyperactivity disorder, migraine, stroke, ischemia or neurodegenerative disease (e.g. Alzheimer's disease, Parkinson's disease, glaucoma or macular degeneration). In addition, compounds that modulate ion channels can be used for treating of cardiovascular diseases (e.g. congestive heart failure, arrhythmia, high blood pressure or restenosis), metabolic diseases and disorders (e.g. diabetes or obesity), proliferation diseases and cancers, or hormonal disorders (e.g. polycystic ovarian syndrome or alopecia). The ion-x polynucleotides and polypeptides, as well as the ion-x modulators, may also be used in diagnostic assays for such diseases or conditions.

ADMINISTRATION - Administration may be oral, intravenous, cutaneous, subcutaneous, nasal, intramuscular or intraperitoneal. Dosage is 0.5 mg/kg of body weight - 500 mg/kg of body weight, preferably 1-50 mg/day.

EXAMPLE - To isolate cDNA clones encoding full length ion channel proteins, DNA fragments corresponding to a portion of nucleic acids having 623, 573, 201, 744, 445, 701, 562, 596, 640, 691, 632, 553, 188, 560, 625, 679, 598, 457 or 550 bp, or their complementary nucleotide sequence, was used as probes for hybridization screening of a phage, phagemid or plasmid cDNA library. The DNA fragments were amplified by PCR. Amplification was performed using for the following program: 95degreesC for 15 seconds, 52degreesC for 30 seconds and 72degreesC for 90 seconds; repeated for 25 cycles. The amplified product was separated from the plasmid by agarose gel electrophoresis, and purified by Qiaquick gel extraction kit. A lambda phage library containing cDNAs cloned into lambda ZAPII phage-vector was plated with E.coli XL-1 blue host, on 15 cm LB-agar plates at a density of 50000 pfu per plate, and grown overnight at 73degreesC. cDNA containing plasmids (pBluescript SK-) were rescued from the isolated phages by in vivo excision by culturing XL-1 blue cells co-infected with isolated phages and with the Excision helper phage. About three positive colonies were selected and grown up in LB medium. Plasmid DNA was isolated from the three clones. The clones were sequenced directly using an ABI377 fluorescence-based sequencer and the ABI PRISM Ready Dye-Deoxy Terminator kit with Taq FSTM polymerase. Generally, sequence reads of up to about 700 bp were obtained. (126

pages)

L7 ANSWER 72 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-05429 BIOTECHDS
TITLE: New mutated eukaryotic initiation factor 2 alpha kinase 3 genes and polypeptides in patients with Wolcott-Rallison syndrome, useful for preventing or treating e.g. diabetes, osteoporosis, arthritis or mental retardation;
involving vector-mediated gene transfer for expression in host cell, for use in drug screening, disease diagnosis, prevention, therapy and gene therapy
AUTHOR: JULIER C; DELEPINE M; NICOLINO M
PATENT ASSIGNEE: INSERM INST NAT SANTE and RECH MEDICALE; CENT NAT GENOTYPAGE
PATENT INFO: WO 2001090371 29 Nov 2001
APPLICATION INFO: WO 2000-IB1153 23 May 2000
PRIORITY INFO: EP 2000-402707 2 Oct 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-122021 [16]
AN 2002-05429 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - An isolated variant of a mammal genomic sequence of the gene coding for the translation initiation factor 2 alpha kinase 3 (EIF2AK3), having a 1115 residue amino acid sequence (I), fully defined in the specification, is new.
DETAILED DESCRIPTION - An isolated variant of a mammal genomic sequence of the gene coding for the translation initiation factor 2 alpha kinase 3 (EIF2AK3), having a 1115 residue amino acid sequence (I), fully defined in the specification, is new. The presence of the variant sequence in a mammal is capable of inducing the Wolcott-Rallison syndrome (WRS), or affects the risk of onset or progression of diabetes and/or pathology related to WRS.
INDEPENDENT CLAIMS are also included for the following: (1) complementary sequence of the novel variant nucleic sequence; (2) a polypeptide encoded by the novel variant nucleic sequence, characterized in that its amino acids sequence presents at least one point variation compared to the sequence (I) of EIF2AK3; (3) an isolated nucleic acid sequence comprising: (a) a fragment of nucleic acid sequence cited above comprising at least 12 bases; or (b) a nucleic sequences capable of hybridizing specifically with (a);(4) cloning and/or expression vector containing the nucleic acid sequence of (3); (5) a host cell transformed by the vector of (4); (6) a mammal, except man, comprising the cell of (5); (7) a method of producing a recombinant polypeptide by culturing the

transformed cells and allowing the expression of the recombinant polypeptide and recovering the polypeptide; (8) a recombinant or synthetic polypeptide obtained by the method of (7); (9) mono- or polyclonal antibodies, or their fragments, chimeric or immunoconjugated antibodies, which are capable of specifically recognizing the polypeptide; (10) a method for screening RNA, cDNA or genomic DNA contained in a biological sample or in libraries by using the nucleic sequence of (3); (11) a method for determining an allelic variability or a loss of heterozygosity by using the EIF2AK3 variant nucleic acid sequence or the nucleic acid of (3); (12) a method for diagnosing diabetes and/or a pathology related to WRS or correlated with an abnormal expression of the EIF2AK3 polypeptide; (13) a method for determining if a subject is at decrease or increased risk of having diabetes and/or a pathology related to WRS; (14) in vitro methods for determining if a subject, where one member of his family is affected by the WRS, is at risk of having WRS; (15) a kit for determining if a subject is at decreased or increased risk of having diabetes and/or pathology related to the WRS, comprising at least one pair of primers capable of amplifying a fragment of genomic DNA or RNA encoding the protein EIF2AK3 and suspected of containing a polymorphism associated to a decreased or increased risk of having diabetes and/or pathology related to the WRS, where the primers comprise the nucleic acid of (3); (16) a method for selecting a chemical or biochemical compound capable of interacting, directly or indirectly, with the EIF2AK3 protein, and/or allowing the expression or the activity of the EIF2AK3 protein to be modulated, characterized in that it uses the cell of the mammal cited above or the polypeptide cited above; and (17) a compound selected by the method of (16).

BIOTECHNOLOGY - Preferred Nucleic Acid: The diabetes and/or pathology related to WRS is selected from type 1 diabetes , type 2 diabetes, the others forms of diabetes, osteoporosis, arthritis, hepatic dysfunction, nephropathies and other renal dysfunction and mental retardation. The diabetes and/or pathology related to WRS is linked to a major decrease of pancreatic beta-cells or its integrity. The diabetes and/or pathology related to WRS results from the alteration of the control, which is exerted by EIF2AK3 on a specific protein from the pancreas and/or from the chondrocytes, the control, if normally exerted, insuring the adequate development and function of these organs. The variant sequence comprises a 4116, 612, 1896, 1595, 1257, 4375, 1243, 1608, 1295, 3794, 828, 1222 or 3348 base pair sequence, all fully defined in the specification, or their fragment, provided the isolated variant nucleic sequence is not the sequence having a 4325 base pair sequence (II), fully defined in the specification. The protein EIF2AK3 encoded by the variant sequence presents at least one point variation compared to (I). The protein EIF2AK3 encoded by the variant sequence presents a

premature termination or at least one point variation in the catalytic domain residues 576-1115 of the protein EIF2AK3 having the sequence (I). The isolated variant nucleic sequence comprises an insertion of a T at position 1103, or a G to A transition at position 1832 in the sequence (II). The sequence also comprises at least one of the nucleic sequence polymorphisms identified in EIF2AK3 exons and flanking intronic regions, which are defined in the specification in a table showing the estimated haplotype combinations and frequencies, and in a table fully described in the specification and showing EIF2AK3 less frequent polymorphisms (where additional variants were identified, which occurred in one or two alleles out of the 190 characterized. The sequence is chosen from a human nucleic sequence. The isolated nucleic acid sequence is characterized in that it encodes the EIF2AK3 polypeptide. The nucleic acid sequence of (3) is a primer or a probe. The nucleic acid sequence comprises any of 90 sequences fully defined in the specification, e.g.: (1) ggggcataac ctaatttgag c; (2) aaaagactga tgggaatgac; or (20) caactcccat agccctttgc. Preferred Polypeptide: The polypeptide is characterized in that it comprises at least one of the amino acid variation as listed in the column amino acid in a table fully described in the specification, where the table shows polymorphisms identified in EIF2AK3 exons and flanking intronic regions. Preferred Vector: The vector is characterized in that it comprises the elements allowing the expression and/or secretion of the sequences in a host cell. Preferred Cell: The cell is an eukaryotic or prokaryotic cell. Preferred Method: In the method of (12), diagnosing diabetes and/or pathology related to WRS or correlated with an abnormal expression of the EIF2AK3 polypeptide is characterized in that one or more antibodies is(are) brought into contact with the biological material to be tested, under conditions allowing the possible formation of specific immunological complexes between the polypeptide and the antibody or antibodies, and in that the immunological complexes possibly formed are detected. Method (13) comprises: (a) collecting a biological sample containing genomic DNA or RNA from the subject; (b) determining on at least one gene allele or RNA encoding the protein EIF2AK3, the sequence or its length, of a fragment of the DNA or RNA susceptible of containing a polymorphism associated to a decrease or increased risk of having diabetes and/or pathology related to WRS, where the fragment can be amplified by polymerase chain reaction with the set of primers cited above; and (c) observing whether or not the subject is at decrease or increased risk of having diabetes and/or pathology related to WRS by observing if the sequence of the fragment of DNA or RNA contains a polymorphism associated with a decreased or increased risk of having pathology related to WRS, where the presence of the polymorphism indicates that the subject is at decrease or increased risk of having diabetes and/or pathology related to WRS. In method (14), determining if a subject, where one member of his family is affected by the WRS, is at risk of having WRS comprises: (a)

collecting a biological sample containing genomic DNA or RNA from the subject; (b) determining on the sequence of both alleles of the EIF2AK3 gene, the sequence or its length, of a fragment of the DNA or RNA susceptible of containing a polymorphism associated to the risk of having WRS, where fragment can be amplified by polymerase chain reaction with a set of primers cited above; and (c) observing if the subject is at risk of having WRS by observing if for both alleles, the sequence of the fragment of DNA or RNA carry a mutation associated to a risk of having WRS, where the presence of the mutation indicates the subject is at risk of having WRS. The diagnosis of the risk of having the WRS is characterized in that the polymorphism associated to the risk of having WRS in step (b) is the presence of the mutation corresponding to an insertion of a T at position 1103, or a G to A transition at position 1832 in (II). The presence of the mutation on each of the EIF2AK3 gene allele of the subject indicates that the subject is at risk of having WRS. The method may also comprise: (a) collecting a biological sample containing genomic DNA or RNA from the family member affected by the WRS and from the subject; and (b) determining if the family member is affected by the WRS and the subject present an allelic identity by comparing polymorphic markers, which are positioned close to or included in the EIF2AK3 gene, the genotype identity between the family member affected by the WRS and the subject indicates the subject is at risk of having WRS. Furthermore, the method involves: (a) collecting a biological sample containing genomic DNA or RNA from the family member affected by the WRS and from the subject; (b) determining on the both EIF2AK3 gene alleles of the family member, the sequence of a fragment of DNA or RNA susceptible of containing a polymorphism associated to the risk of having WRS, where the fragment can be amplified by polymerase chain reaction with the set of primers cited above; (c) determining if the mutation of the sequence of the fragments responsible of the WRS affection identified in step (b) is present on the same fragment of both the EIF2AK3 gene alleles of the subject; and (d) observing if the subject is at risk of having WRS by observing if the sequence of the fragment on the both EIF2AK3 gene alleles of the subject contains the same mutation as identified in step (b) for the family's member, the presence of the mutation on the both alleles indicates the subject is at risk of having WRS. The sequence or its length, of a fragment of DNA or RNA suspected of containing the polymorphism is obtained in step (b) by determining the size of and/or sequencing the amplified products obtained after polymerase chain reaction (PCR), eventually after a step of reverse transcription. The method further comprises a second method for assaying a biological sample from the subject for levels of at least an additional marker associated with the decreased or increased risk of developing diabetes and/or pathology related to the WRS, the presence of a significantly level of the at least one marker allowing to confirm if the subject is at decreased or increased risk of

developing the diabetes and/or pathology related to the WRS. Preferred Kit: The kit further comprises a means for assaying a biological sample from the subject for levels of at least an additional marker associated with a decreased or increased risk of having diabetes and/or pathology related to the WRS. The comprises at least one pair of primers capable of amplifying a fragment of genomic DNA containing a polymorphic marker, which is positioned close to or included in the EIF2AX3 gene. Preferred Compound: The compound is characterized in that it allows: (a) a modulation of the level of EIF2AK3 protein expression; and/or (b) an increase of pancreatic beta-cells or their integrity; and/or (c) the prevention or treatment of diabetes and/or pathology related to the WRS. The compound is chosen from the antibody, the polypeptide, the vector, or the sense or anti-sense nucleic sequence cited above.

ACTIVITY - Antidiabetic; Osteopathic; Antiarthritic; Hepatotropic; Nephrotropic; Nootropic. No biological data is given.

MECHANISM OF ACTION - Gene therapy; eukaryotic translation initiation factor 2 alpha kinase 3 modulator.

USE - The EIF2AK3 nucleic acid variant is useful for the production of a recombinant or synthetic polypeptide, and for screening compounds capable of modulating EIF2AK3. The nucleic acid is also useful for screening or diagnosing the diseases cited below. The nucleic acid of (3) may be used as sense or anti-sense oligonucleotide. The nucleic acid may also be used as a primer or a probe, for detecting and/or amplifying a nucleic acid sequence. The compound is useful as a medicament, particularly for preventing and/or treating diabetes and/or pathology related to WRS, e.g. type 1 diabetes, type 2 diabetes, the others forms of diabetes, osteoporosis, arthritis, hepatic dysfunction, nephropathies or other renal dysfunction, or mental retardation. The cell the mammal or the polypeptide is useful for studying the expression or the activity of the EIF2AK3 protein, and the direct or indirect interactions between the EIF2AK3 protein and chemical or biochemical compounds, which may be involved in the activity of the EIF2AK3 protein. The cell or polypeptide is also useful for screening chemical or biochemical compounds capable of interacting directly or indirectly with the EIF2AK3 protein, and/or capable of modulating the expression or the activity of the EIF2AK3 protein. (All claimed).

ADMINISTRATION - None given.

EXAMPLE - Mutation screening was performed in a Wolcott-Rallison syndrome (WRS) index patient and his two parents, with normal Caucasian individual used as a control, by direct sequencing of the coding regions of the cDNA on reverse transcriptase-polymerase chain reaction (RT-PCR) amplified product, and on a WRS2 index patient and his father, with a normal Caucasian individual used as a control, by sequencing coding regions of the gene on PCR-amplified genomic DNA.

Co-segregation of the mutation identified in WRS1 family with WRS was confirmed on genomic DNA by PCR-restriction fragment length polymorphism (RFLP) method using primers PEK1 and PEK2 followed by a restriction enzyme digest with AflII. After digest, the normal allele gave 302 and 35 base pair fragments (presence of the restriction enzyme site), while the mutant allele gave a 337 base pair fragment (no site), which were resolved by agarose gel electrophoresis. PEK1: CTGACTGGAAAGTTATGG and PEK2: AAAAGACTGATGGGAATGAC.(93 pages)

L7 ANSWER 73 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-07411 BIOTECHDS
TITLE: New compositions comprising rabbit paraoxonase 3 genes or polypeptides, useful for treating or preventing sepsis, endotoxemia, oxidative damage in atherosclerosis or neuropathy, or chemical toxicity;
vector-mediated recombinant enzyme gene transfer and expression in host cell, antibody, drug screening, reverse transcription-polymerase chain reaction and DNA primer for disease diagnosis and genetherapy
AUTHOR: LA DU B N; DRAGANOV D I; STETSON P; WATSON C E
PATENT ASSIGNEE: UNIV MICHIGAN
PATENT INFO: WO 2001090336 29 Nov 2001
APPLICATION INFO: WO 2000-US16126 19 May 2000
PRIORITY INFO: US 2000-574377 19 May 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-089928 [12]
AN 2002-07411 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - A composition consisting of a nucleic acid sequence (I) comprising at least a portion of a gene encoding rabbit paraoxonase-3 (PON3), is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) compositions consisting of a nucleic acid comprising a PON3 variant, a polypeptide encoded by (I), or a biological active rabbit PON-3 polypeptide or its fragment; and (2) a method comprising providing a biologically active PON3 polypeptide or its fragment, a host and a delivery system, and administering the biologically active rabbit PON3 polypeptide or its fragment to the host using the delivery system.
WIDER DISCLOSURE - Also disclosed are the following: (1) vectors comprising the PON-3 gene; (2) host cells containing the vectors or constructs; (3) fusion proteins incorporating all or part of rabbit PON3; (4) mutant or variant forms of PON3; and (5) antibodies that

specifically recognize PON3.

BIOTECHNOLOGY - Preferred Composition: The nucleic acid comprises a fully defined sequence of 1164 bp given in the specification, or a portion of the defined sequence containing a truncation mutation. The polypeptide comprises a fully defined sequence of 355 amino acids given in the specification. The polypeptide may also comprise a non-rabbit PON3 polypeptide, preferably human PON3 polypeptide. Preferred Method: The method further comprises delivering a second PON polypeptide to the host using the delivery system, where the second PON is provided in a mixture with the PON3 polypeptide. The second PON is PON1 or PON2. The host comprises a host suspected of having sepsis or susceptible to sepsis.

ACTIVITY - Antibacterial; immunosuppressive; antiarteriosclerotic; antidiabetic.

MECHANISM OF ACTION - Gene therapy.

USE - The PON-3 genes, variants, homologs, mutants or fusion proteins are useful for treating or preventing sepsis, endotoxemia, oxidative damage in atherosclerosis, neuropathy, microangiopathy in systemic diseases such as diabetes, and chemical toxicity (e.g. in the treatment of toxic side effects of HMG-CoA reductase inhibitors). PON3 may also be used as a target for screening drugs that alter lactone production, metabolism and clearance. PON3 peptides may be used as immunogen to generate antibodies that recognize rabbit PON3. These antibodies may be used to detect PON3 in a biological sample.

ADMINISTRATION - Compositions may be administered orally, parenterally (e.g. intravenous, subcutaneous, intramuscular, intramedullary, intrathecal, intraventricular, intraperitoneal or intranasal), systemically or locally. Dosage is 0.1-100000 microg, up to a total dose of 1 g.

EXAMPLE - The nucleotide sequence or rabbit paraoxonase-3 (PON3) was obtained by reverse transcriptase- polyacrylamide gel electrophoresis (RT-PCR) of total liver RNA isolated from New Zealand white rabbits. RT-PCR was performed using primers for the human PON3, Px3-6 and Px3-17. RT-PCR products were separated on a 1% agarose gel, and bands of the expected size were excised and sequenced. Based on the obtained sequence, primers RPx3-4 and Rpx3-1 were designed and used for the amplification of the 5' and 3' ends of rabbit PON3 cDNA. Based on the results from sequencing these fragments, new primers RPx3-5 and Rp3-6 were designed and used to amplify the full-length cDNA. The PCR product was cloned in pCRII vector and sequenced. The rabbit PON3 clone has a fully defined sequence of 1164 base pairs given in the specification, with a deduced 355-amino acid sequence also given in the specification. Px3-6 5'-GGCATAGAACTGTTCTGGTCCAAGAACC-3'; Px3-17 5'GCTTCTGAAGATATTGATATACTCCCCAGTGGGC-3'; RPx3-4 5'-CTCATCTGGTGCAAAGTTTGG-3'; Rpx3-1 5'-ACAACAACGCTCTCTTGTAC-3'; RPx3-5 5'-

ATCGGAATTCCATGGCGAAGCTCCTGC-3; Rp3-6 5'-
AGGCCTCGAGCTGGAGACTAGAGCAC-3'
.(94 pages)

L7 ANSWER 74 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-06671 BIOTECHDS
TITLE: Novel polynucleotide which down regulates expression of Ets-related gene, useful for treating cancer, diabetic retinopathy, macular degeneration, arthritis, psoriasis, verruca vulgaris and Sturge Weber syndrome; vector-mediated gene transfer, expression in mammal host cell, antisense oligonucleotide or ribozyme for gene therapy and disease diagnosis
AUTHOR: JARVIS T; VON CARLOWITZ I; MCSWIGGEN J A; MCLAUGHLIN F; RANDI A M
PATENT ASSIGNEE: RIBOZYME PHARM INC; GLAXO GROUP LTD
PATENT INFO: WO 2001088124 22 Nov 2001
APPLICATION INFO: WO 2000-US15866 16 May 2000
PRIORITY INFO: US 2000-572021 16 May 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-082995 [11]
AN 2002-06671 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - A nucleic acid molecule (I) which down regulates expression of an Ets-related gene (ERG), is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an expression vector (II) comprising a nucleic acid sequence encoding (I) in a manner which allows expression of (I); and (2) a mammalian cell (III) including (I) or (II).
WIDER DISCLOSURE - Also disclosed are: (1) a method for producing a class of nucleic acid-based gene inhibiting agents which exhibit a high degree of specificity for the RNA of a desired target; (2) a pharmaceutically acceptable formulation of (I); and (3) a composition prepared for storage or administration which includes (I) in a pharmaceutically acceptable carrier or diluent.
BIOTECHNOLOGY - Preferred Polynucleotide: (I) is an enzymatic nucleic acid molecule, an antisense nucleic acid molecule or a DNAzyme. The antisense nucleic acid molecule or the binding arm of the enzymatic nucleic acid molecule comprises a sequence complementary to 2145 sequences defined in the specification such as GCGCGUGUCCGCGCCCG or GCGUGCCUUGGCCGUGC. The enzymatic nucleic acid molecule comprises 3162 sequences fully defined in the specification such as

CGGGCGCGCUGAUGAGXCGAAACACGCGC. The antisense nucleic acid molecule
comprises 20 sequences fully defined in the specification such as BucccgccTSTSTSGSGSCSCSASCSacugcauB. The enzymatic nucleic acid molecule is in a hammerhead (HH) motif, hairpin, hepatitis DELTA-virus, group I intron, VS nucleic acid, amberzyme, zinzyme, RNAse P nucleic acid motif, Inozyme motif, or G-cleaver motif. The HH motif comprises sequences complementary to 491 sequences fully defined in the specification such as GCGCGUGUCCGCGCCCG. The Inozyme motif comprises sequences complementary to
661 sequences fully defined in the specification such as CGCGUGUCCGCGCCCGC. The G-cleaver motif comprises sequences complementary
to 208 sequences fully defined in the specification such as CCGCGCGUGUCCGCGCC. The DNAzyme, zinzyme and amberzyme comprises sequences
complementary to sequences fully defined in the specification. (I) comprises between 12-100 bases, preferably 14-24 bases complementary to the RNA of ERG gene. (I) comprises at least one 2'-sugar modification, a nucleic acid base modification or a phosphate backbone modification. (I) comprises a cap structure which is at the 5'-end or 3'-end or both the 5'-end and the 3'-end. (I) comprises at least five ribose residues, at least ten 2'-O-methyl modifications, and a 3'-end modification. (I) further comprises a phosphorothioate core with both 3' and 5'-end modifications, where the 3' and/or 5'-end modification is 3'-3' inverted abasic group. (I) further comprises phosphorothioate linkages on at least three of the 5' terminal nucleotides. Preferred Vector: (II) further comprises a sequence for an antisense nucleic acid molecule complementary to the RNA of ERG gene or a sequence encoding enzymatic nucleic acid molecule complementary to the RNA of ERG gene. (II) comprises sequence encoding at least two of the nucleic acid molecules, which may be same or different. Preferred Cell: (III) is a human cell.

ACTIVITY - Cytostatic; antitumor; antidiabetic; ophthalmological; antiarthritic; antipsoriatic; virucide; osteopathic; vulnerary.

MECHANISM OF ACTION - Down regulator of ERG gene expression (claimed); gene therapy. The ability of Ets-related Geneblocs (I) to decrease Ets-related gene (Erg) mRNA and protein levels in human umbilical vein endothelial cells (HUVEC) was tested. In order to clearly define Erg regulated genes in endothelial cells, antisense oligonucleotides were designed. GeneBlocs were modified RNA oligonucleotides which were less susceptible to degradation, had reduced toxicity and increased target binding affinity than traditional antisense oligonucleotides. GeneBlocs were designed to target region of human Erg which was not homologous to other closely related Ets family members Ets-1, Ets-2, Fli-1 or Nerf, which were also

expressed in endothelial cells. In initial studies 8 Erg GeneBlocs were designed and inhibition of Erg mRNA in HUVEC was assayed using Taqman. The results showed that two GeneBlocs (14573 and 14566) gave greater than 80% inhibition of Erg mRNA levels after 24 hours, when compared to a random scrambled control GeneBloc (11698).

USE - (I) is useful for treating conditions selected from cancer, lymphoma, Ewing's sarcoma, melanoma, tumor angiogenesis, diabetic retinopathy, macular degeneration, neovascular glaucoma, myopic degeneration, arthritis, psoriasis, verruca vulgaris, angiofibroma of tuberous sclerosis, port-wine stains, Sturge Weber syndrome, Kippel-Trenaunay-Weber syndrome, Osler-Weber-rendu syndrome, leukemia, osteoporosis and wound healing. (I) is useful for treating a patient having a condition associated with the level of ERG, by contacting cells of the patient with (I) under conditions suitable for the treatment. The method comprises the use of one or more therapies under conditions suitable for the treatment. Leukemia or tumor angiogenesis is treated by administering (I) to the patient in conjunction with one or more of other therapies such as radiation or chemotherapy treatment. (I) is useful for reducing ERG activity in a cell, by contacting the cell with (I). (I) is useful for cleaving RNA of ERG gene, by contacting (I) with RNA, in the presence of a divalent cation such as Mg2+ (claimed). (I) is useful for diagnosis of conditions and diseases related to the expression of ERG, and as diagnostic tool to examine genetic drift and mutations within diseased cells or to detect the presence of ERG RNA in a cell. (I) is useful for specifically targeting genes that share homology with ERG gene or ERG fusion genes.

ADMINISTRATION - (I) is administered by intravascular, intramuscular, subcutaneous, oral, topical, systemic, ocular, intraperitoneal, intrathecal, or rectal route, or by aerosol inhalation or joint injection at a dose of 0.1-100 mg/kg/day.

EXAMPLE - Chemical synthesis and purification of ribozymes and antisense for efficient cleavage and/or blocking of Ets-related gene (ERG) RNA was as follows: Ribozymes and antisense constructs were designed to anneal to various sites in the RNA message. The binding arms of the ribozymes were complementary to the target site sequences, while the antisense constructs were fully complementary to the target site sequences. The ribozymes and antisense constructs were chemically synthesized. The method of synthesis used followed the procedure for normal RNA synthesis, and made use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. Ribozymes and antisense constructs were also synthesized from DNA templates using bacteriophage T7 RNA polymerase. Ribozymes and antisense constructs were purified by gel electrophoresis or high pressure liquid chromatography (HPLC) and were resuspended in water. (149 pages)

L7 ANSWER 75 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-07398 BIOTECHDS
TITLE: Novel G-protein coupled receptors and polynucleotides useful for diagnosis, treatment and prevention of disorders of cell proliferation, neurological, cardiovascular, metabolic disorders and viral infections;
vector-mediated gene transfer, expression in host cell, antibody, transgenic animal, cDNA library, database, computer bioinformatic software and high throughput screening for recombinant protein production, drug screening anddisease gene therapy
AUTHOR: PATTERSON C; LU D A M; THORNTON M; LU Y; TRIBOULEY C M; GRAUL R; KHAN F A; GANDHI A R; WALIA N K; NGUYEN D B; YUE H; HAFALIA A; ELLIOTT V S; LAL P; REDDY R; KALLICK D A; TANG T Y; AU-YOUNG J
PATENT ASSIGNEE: INCYTE GENOMICS INC
PATENT INFO: WO 2001087937 22 Nov 2001
APPLICATION INFO: WO 2000-US16285 18 May 2000
PRIORITY INFO: US 2000-208861 2 Jun 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-089844 [12]
AN 2002-07398 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - An isolated polypeptide (I) of G-protein coupled receptor (GCREC) having a 372, 337, 346, 432, 240, 271, 276 or 408 residue amino acid sequence (S1), fully defined in the specification, a naturally occurring polypeptide comprising at least 90 % identity to (S1), a biologically active fragment of PP, and an immunogenic fragment PP, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated polynucleotide (II) encoding (I); (2) a recombinant polynucleotide (III) comprising a promoter sequence operably linked to (II); (3) a cell (IV) transformed with (III); (4) a transgenic organism (V) comprising (III); (5) preparation of (I), comprising culturing (IV) under expression conditions, and recovering the polypeptide; (6) an isolated antibody (VI) which specifically binds to (I); (7) an isolated polynucleotide (VII) comprising at least 60 contiguous nucleotides of (II); (7) detecting (M1) a target polynucleotide in a sample, where the target polynucleotide having a sequence of (II), comprising: (a) amplifying the target polynucleotide or their fragment using polymerase chain reaction; (b)

detecting the amplified target polynucleotide or their fragment; and (c) optionally if present, determining the amount; (8) a composition (C1) comprising (I) or agonist of (I) identified by using (I), and an excipient; (9) a composition (C2) comprising antagonist of (I) identified by using (I) and an excipient; (10) a composition (C3) comprising (VI) and an excipient; (11) an polyclonal antibody (VIII) produced by using (I); (12) a monoclonal antibody (IX) produced by using (I); and (13) a composition (C4) comprising (VIII) or (IX) and a suitable carrier.

WIDER DISCLOSURE - A polynucleotide capable of hybridizing to (II) under stringency conditions, is disclosed as new.

BIOTECHNOLOGY - Preparation: (I) is prepared by culturing (IV) under conditions suitable for expression of (I), and recovering (I) so expressed. (VI) is produced by screening a Fab expression library, or a recombinant immunoglobulin library. Preferred Polynucleotide: (II) is preferably from a polynucleotide (P1) comprising a 2444, 1014, 1083, 1740, 3002, 965, 1617 or 1227 base pair sequence (S2), fully defined in the specification, a naturally occurring polynucleotide (P2) comprising a sequence at least 90 % identical to (S2), a polynucleotide (P3) complementary to polynucleotide of (P1) or (P2), and an RNA equivalent of (P1)-(P3). Preferred Antibody: (VI) is a chimeric antibody, single chain antibody, a Fab fragment, a F(ab')2 fragment or a humanized antibody.

ACTIVITY - Antiarteriosclerotic; Antiinflammatory; Hepatotropic; Antipsoriatic; Cytostatic; Anti-convulsant; Nootropic; Neuroprotective; Antiparkinsonian; Neuroleptic; Antianemic; Hypotensive; Cardiant; Antidiabetic; Antiulcer; Antidiarrheic; Laxative; Anti-HIV (human immunodeficiency virus); Anti-allergic; Dermatological; Antiasthmatic; Antigout; Antirheumatic; Antiarthritic; Immunosuppressive; Tranquilizer; Vulnerary; Ophthalmological; Anorectic; Osteopathic; Anti-bacterial; Fungicide; Virucide; Antiparasitic; Protozoacide; Nephrotropic. No biological data is given.

MECHANISM OF ACTION - Gene therapy; (I) modulator.

USE - (VII) is useful as a probe for detecting a target polynucleotide in a sample, where the target polynucleotide having a sequence of (II). (I) is useful for screening a compound for effectiveness as an agonist or antagonist of (I). The method comprises exposing a sample comprising (I) to a compound and detecting agonist or antagonist activity in the sample. (C1) and (C2) are useful for treating a disease or condition associated with decreased and overexpression of functional GCREC, respectively. (I) is also useful for screening a compound that specifically binds to (I). (I) is further useful for screening for a compound that modulates the activity of (I). (II) is useful for screening (M5) a compound for effectiveness in altering expression of a target polynucleotide. (VII) is useful as a probe for assessing toxicity of a test compound. (VI) is useful for

diagnostic test for a condition or disease associated with the expression of GCREC in a biological sample. (I) is useful for preparing a polyclonal antibody with the specificity of (VI). (I) is further useful for making a monoclonal antibody with the specificity of (VI). (VI) is also useful for detecting a (I). (All claimed). (I) is useful for diagnosing, treating, preventing disorders which include cell proliferative disorders such as arteriosclerosis, hepatitis, myelofibrosis, psoriasis and cancer including adenocarcinoma, leukemia, lymphoma; neurological disorders such as epilepsy, ischemic cerebrovascular disease, Alzheimer's disease, Pick's disease, dementia, Parkinson's disease, ataxias, multiple sclerosis, bacterial and viral meningitis, Creutzfeldt-Jakob disease, schizophrenic disorders, amnesia; cardiovascular disorders such as arteriovenous fistula, atherosclerosis, hypertension, vascular tumors, myocardial infarction, hypertensive heart disease, infective endocarditis, cardiomyopathy, myocarditis; gastrointestinal disorders such as dysphagia, peptic esophagitis, anorexia, nausea, emesis, peptic ulcer, cholelithiasis, diarrhea, constipation, acquired immunodeficiency syndrome (AIDS), hepatic encephalopathy; autoimmune/inflammatory disorders such as Addison's disease, allergies, spondylitis, amyloidosis, anemia, asthma, contact dermatitis, Crohn's disease , diabetes mellitus, emphysema, Goodpasture's syndrome, gout, Graves' disease, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, uveitis, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma; metabolic disorders such as diabetes, obesity and osteoporosis; or viral infections such as infection caused by viral agent classified as adenovirus, arenavirus, bunyavirus; and also in the assessment of effects of exogenous compounds on the expression of nucleic acid sequence of GPCR.

ADMINISTRATION - (II) is administered by a adenovirus-, or herpes-based gene therapy delivery system. C1, C2 or C3 is administered through oral, intravenous, intramuscular, intraarterial, intramedullary, intrathecal, intraventricular, pulmonary, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual or rectal routes. Dosage is 0.1-100000 micro-g upto a total dose of 1 g.

EXAMPLE - Incyte cDNAs were derived from cDNA libraries described in the LIFESEQ GOLD (RTM) database. Some tissue were homogenized and lysed in guanidinium isothiocyanate, while other were homogenized and lysed in phenol or in a suitable mixture of denaturants, such as TRIZOL (RTM). The resulting lysates were centrifuged over CsCl cushion or extracted with chloroform. RNA was precipitated from the lysates with either isopropanol or sodium acetate and ethanol, or by other routine methods. Phenol extraction and precipitation of RNA were repeated as necessary to increase RNA purity. Optionally, RNA was treated with DNase. For most

libraries, poly(A)+RNA was isolated using oligo d(T)-coupled paramagnetic particles (Promega), OLIGOTEX (RTM) latex particles. Alternatively, RNA was isolated directly from tissue lysates using other RNA isolation kits, e.g. the POLY(A)PURE (RTM) mRNA purification kit. cDNA was obtained from RNA by Stratagene or synthesized and cDNA libraries were constructed with UNIZAP (RTM) vector system or SUPERSCRIPT (RTM) plasmid system. Reverse transcription was initiated using oligo d(T) or random primers. Synthetic oligonucleotide adapters were ligated to double stranded cDNA, and the cDNA was digested with the appropriate restriction enzyme or enzymes. For most libraries, the cDNA was size-selected (300-1000 base pair) using SEPHACRYL S1000 (RTM), SEPHAROSE CL2B (RTM), or SEPHAROSE CL4B (RTM)
column chromatography or preparative agarose gel electrophoresis . cDNAs were ligated into compatible restriction enzyme sites of the polylinker of a suitable plasmid e.g. pBLUESCRIPT (RTM) plasmid. Recombinant plasmids were transformed into competent Escherichia coli cells including XL1-Blue. Then, plasmids were recovered from host cells by in vivo excision using the UNIZAP (RTM) vector system and were purified. Following precipitation, plasmids were resuspended in 0.1 ml of distilled water and stored, with or without lyophilization at 4 degrees C. Alternatively, plasmid DNA was amplified from host cell lysates using direct link polymerase chain reaction (PCR) in a high-throughput format. Host cell lysis and thermal cycling steps were carried out in a single reaction mixture. Samples were processed and stored in 384-well plates, and the concentration of amplified plasmid DNA was quantified fluorometrically using PICOGREEN (RTM) dye and a FLUOROSKAN II (RTM) fluorescence scanner. Incyte cDNA recovered in plasmids were sequenced. The Incyte cDNA sequences or their translations were then queried against a selection of public databases such as the GenBank primate, rodent, mammalian, vertebrate, and eukaryotic databases, and BLOCKS, PRINTS, DOMO, PRODOM, and hidden Markov model (HMM)-based protein family databases such as PFAM. The queries were performed using programs based on BLAST, FASTA, BLIMPS, and HMMER. The Incyte cDNA sequences were assembled to produce full length polynucleotide sequences. Alternatively, GenBank cDNAs, GenBank expressed sequence tags (EST), stitched sequences, stretched sequences, or Genscan-predicted coding sequences were used to extend Incyte cDNA assemblages to full length. Assembly was performed using programs based on Phred, Phrap, and Consed, and cDNA assemblages were screened for open reading frames using programs based on GeneMark, BLAST, and FASTA. The full length polynucleotide sequences were translated to derive the corresponding full length polypeptide sequences.(135 pages)

L7 ANSWER 76 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-06964 BIOTECHDS

TITLE: New human monoclonal antibodies specific for dendritic cells, useful for inhibiting growth or inducing cytolysis of a dendritic cell and treating or preventing a dendritic cell mediated disease, e.g., autoimmune disorders; dendritic cell-specific monoclonal antibody preparation by hybridoma cell culture, transgenic animal and PAGE for disease therapy and prevention

AUTHOR: DEO Y M; KELER T
PATENT ASSIGNEE: MEDAREX INC

PATENT INFO: WO 2001085798 15 Nov 2001
APPLICATION INFO: WO 2000-US15114 8 May 2000
PRIORITY INFO: US 2000-230739 7 Sep 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-089788 [12]
AN 2002-06964 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - Isolated human monoclonal antibodies (I), or their antigen binding portions that specifically bind to dendritic cells, are new.

DETAILED DESCRIPTION - The isolated human monoclonal antibody (I) or its antigen binding portion has one or more of the following characteristics: (a) a binding affinity constant to a dendritic cell of at least about 10 7 M-1; (b) the ability to opsonize a dendritic cell; (c) the ability to internalize after binding to dendritic cells; or (d) the ability to activate dendritic cells. The isolated human monoclonal antibody or its antigen binding portion may also have any of the following characteristics: (a) mediates cytolysis of dendritic cells in the presence of human effector cells; or (b) inhibits growth of dendritic cells. (I) comprises a variable light chain having the sequence comprising 107 amino acids fully defined in the specification, and a variable heavy chain having the sequence comprising 116 amino acids fully defined in the specification. Furthermore, (I) or its antigen binding portion, binds to and blocks the human mannose receptor on dendritic cells. The antibody has a molecular weight of 36-40 kD as measured by polyacrylamide gel electrophoresis (PAGE) on human dermal dendritic cells, human epidermal dendritic cells, and dendritic cells derived from cynomolgus macaques. INDEPENDENT CLAIMS are also included for the following: (1) a hybridoma comprising a B cell obtained from a transgenic non-human animal having a genome comprising a human heavy chain transgene and a light chain transgene fused to an immortalized cell, where the hybridoma produces a detectable amount of (I); (2) a transgenic non-human animal, which expresses (I), where the transgenic non-human animal has a genome comprising a human heavy chain transgene and a human light chain transgene; (3) producing (I); (4) bispecific molecules comprising: (a) at least one first binding specificity for dendritic cells and a second binding specificity for an

Fc receptor; or (b) at least one first binding specificity for dendritic cells and a second binding specificity for an antigen on a target cell; (5) compositions comprising: (a) the isolated human monoclonal antibody or its antigen-binding portion and a pharmaceutical carrier; or (b) a combination of two or more isolated human antibodies or antigen-binding portions, where each of the antibodies or antigen-binding portions binds to a distinct epitope on a dendritic cell; (6) inhibiting growth of a dendritic cell comprising contacting a dendritic cell with (I) or its antigen-binding portion; (7) inducing cytolysis of a dendritic cell comprising contacting a dendritic cell with (I) or its antigen-binding portion that specifically binds to dendritic cells in the presence of an effector cell, such that cytolysis of the dendritic cell occurs; (8) treating or preventing a dendritic cell mediated disease by administering (I) or its antigen binding portion; (9) detecting the presence of a dendritic cell in a sample comprising: (a) contacting the sample and a control sample, with (I) or its antigen binding portion to allow the formation of a complex between the antibody or its portion and the dendritic cell; and (b) detecting the formation of a complex, where a difference complex formation between the sample compared to the control sample is indicative the presence of dendritic cell in the sample; (10) an expression vector comprising a nucleotide sequence encoding a variable and constant region of the heavy and light chains (I) or its antigen binding portion; (11) targeting an antigen to a dendritic cell in a subject by administering (I) or its antigen binding portion, which is operably linked to an antigen, such that antigen is targeted to the dendritic cell; (12) a molecular complex comprising: (a) at least one binding specificity for a component on the surface of a dendritic cell; and (b) at least one antigen linked to the binding specificity, where the component mediates internalization of the molecular complex when bound by the binding specificity; (13) inducing or enhancing an immune response against an antigen in a subject comprising administering to the subject the molecular complex; (14) immunizing a subject comprising administering to the subject the molecular complex; (15) targeting a cell to a dendritic cell; and (16) preventing binding of a pathogen to human mannose receptor on dendritic cells by contacting (I) or its antigen binding portion with dendritic cells to prevent binding of the pathogen to the cells.

BIOTECHNOLOGY - Preparation: (I) is prepared by a method comprising: (a) immunizing a transgenic non-human animal having a genome comprising a human heavy chain transgene and a human light chain transgene with dendritic cells, such that antibodies are produced by B cells of the animal; (b) isolating B cells of the animal; and (c) fusing the B cells with myeloma cells to form immortal, hybridoma cells that secrete human monoclonal antibodies specific for dendritic cells (claimed). Preferred Antibody: The isolated human antibody or its antigen binding portion has

an isotype consisting of IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgAsec, IgD and IgE, preferably an IgG1kappa. (I) or its antigen binding portion binds to an antigen present of the cell surface of a dendritic cell, particularly to the macrophage mannose receptor and is produced by a hybridoma, which includes a B cell obtained from a transgenic non-human animal having a genome comprising a human heavy chain transgene and a human light chain transgene fused to an immortalized cell. In particular, the hybridoma is selected from A3, A5, A23, A24, A33, B9, B11, B33, B47, C8, C10, C20, C28, C29, C30, C35, E1, E8, E10, E18, E20, E21 and E24. The isolated human antibody or its antigen binding portion is capable of mediating cytolysis of dendritic cells by human effector cells at an IC50 of 1 x 10-7 M or less in vitro. In addition, (I) may be conjugated to a binding specificity for a Fc receptor, a cytotoxin or to an immunomodulatory compound. Moreover, (I) induces cytokine release by dendritic cells or modulates the expression of immunomodulatory receptors on the surface of dendritic cells. The immunomodulatory receptor is selected from CD80 (B7.1), CD86 (B7.2), CD40, and CD54 (ICAM). Preferred Method: In the method of (13), the immune response comprises antibodies that bind to the antigen, or T cells that bind to the antigen as a component of an MHC-I or MHC-II complex. In method (15), targeting a cell to a dendritic cell comprises linking a human monoclonal antibody or its antigen binding portion to the surface of a cell, such that the cell is targeted to a dendritic cell. It may also comprise transfecting a cell with a nucleic acid molecule encoding a human monoclonal antibody or its antigen binding portion, such that the cell expresses the antibody or antigen binding fragment on the surface of the cell, thereby targeting the cell to a dendritic cell. Preferred Molecule: The Fc receptor is a human FcgammaRI or a human Fcalpha receptor. The bispecific molecule binds to the Fc receptor at a site, which is distinct from the immunoglobulin binding site of the receptor. The molecular complex has one or more the binding specificities comprising an antibody consisting of A3, A5, A23, A24, A33, B9, B11, B33, B47, C8, C10, C20, C28, C29, C30, C35, E1, E8, E10, E18, E20, E21 and E24, or their antigen binding fragments. The antigen is selected from a tumor antigen, a microbial antigen, a viral antigen, and an autoantigen. The antigen is chemically linked to the binding specificity or is recombinantly fused to the binding specificity.

ACTIVITY - Immunomodulatory; antiinflammatory; antirheumatic; antiarthritic; neuroprotective; antidiabetic; antianemic; endocrine; dermatological; antithyroid; uropathic; opthalmological; muscular. No supporting data given.

MECHANISM OF ACTION - Dendritic cell modulator. The antibody B11 conjugated to tetanus toxoid (TT) or TT alone was added at various concentrations to dendritic cells. Autologous TT-specific T cells were added to each well containing dendritic cells at 50 000 cells per well. Cells were cultures together for 7 days at 37 degreesC and assayed for

the number of living cells using a 3-(4,5-dimethylthiazol-2 -yl)-2,5-diphenyl tetrazolium bromide (MTT) based assay. The ability to induce dendritic cells to specifically stimulate TT-specific T lymphocytes was compared after exposing cells to TT or antibody B11-TT. The results showed that conjugating TT as a model antigen to B11 leads to more efficient antigen presentation as measured by antigen-specific T cell proliferation. T-cell stimulation index for B11-TT was (approximate values) 3.5, 2.1, 1.4, 1.2 and 1.1 at an antigen concentration of 10, 1, 0.1, 0.01 and 0.001 microg/ml respectively, compared to (approximate values) 1.6, 1.2, 0.9, 1.1 and 1.0 for TT alone at the same antigen concentrations, respectively.

USE - (I) or their antigen-binding fragments are useful for inhibiting growth of a dendritic cell, inducing cytolysis of a dendritic cell, treating or preventing a dendritic cell mediated disease, detecting the presence of a dendritic cell, targeting an antigen to a dendritic cell and preventing binding of a pathogen (a virus or a bacterium) to human mannose receptor on dendritic cells. In particular, (I) may be used to treat, e.g., autoimmune disease or graft versus host disease (all claimed). Furthermore, (I) may also be useful for treating immune system or inflammatory disorders (e.g., rheumatoid arthritis), multiple sclerosis, diabetes mellitus, myasthenia gravis, pernicious anemia, Addison's disease , lupus erythematosus, Reiter's syndrome, and Graves disease.

ADMINISTRATION - Administration is oral, nasal topical (e.g., buccal and sublingual), rectal, vaginal or parenteral (e.g., intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac or intradermal). No dosage given.

EXAMPLE - Human anti-dendritic cell monoclonal antibodies were generated by immunizing the HCO7 strain HuMAb mice with preparations of dendritic cells. Human peripheral blood mononuclear cells (PBMCs) were obtained by density gradient centrifugation of whole blood. Monocytes were isolated by adherence to tissue culture flasks for two hours, and then differentiated into dendritic cells. Cells for immunization were used fresh or stored frozen at -80 degreesC. Mice were immunized every 2-3 weeks. Finally, an intravenous injection of dendritic cells in phosphate buffered saline (PBS) was performed prior to splenectomy. The spleens from responding mice were harvested and dispersed into single cells. To generate hybridomas producing anti-dendritic cell antibodies, splenocytes from mice with plasma containing anti-dendritic cell antibodies were fused with P3X63-Ag8.653 myeloma cells (ATCC CRL 1580) and PEG. Hybridomas were selected by growth in HAT containing media. After hybridomas grew out, each well containing hybridomas was screened for the production of human IgG using an anti-human IgG ELISA. Positive hybridomas were screened for and selected based on the following properties: (1) production of human IgG antibodies, and (2) binding to dendritic cells. The hybridomas

screening human IgG were tested for creativity with various types of blood cells by flow cytometry. Dendritic cells were prepared from adherent mononuclear cells by culturing for 5-7 days in media supplemented with GM-CSF and IL-4. Granulocytes (PMN), monocytes and lymphocytes were obtained from heparanized whole blood. The cells were incubated with hybridoma supernatants from IgG-positive clones at 4C. Several hybridomas that were screened produced human IgG1kappa antibodies that demonstrated reactivity with dendritic cells as assessed by flow cytometry. (95 pages)

L7 ANSWER 77 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2001-07250 BIOTECHDS
TITLE: Novel antisense compound targeted to a nucleic acid molecule encoding human SHP-2 is useful for inhibiting the expression of human SHP-2 and treating diabetes and inflammatory disorders; disease therapy
AUTHOR: Bennett C F; Cowsert L M
PATENT ASSIGNEE: Isis-Pharm.
LOCATION: Carlsbad, CA, USA.
PATENT INFO: WO 2001007655 1 Feb 2001
APPLICATION INFO: WO 2000-US19622 19 Jul 2000
PRIORITY INFO: US 1999-358683 21 Jul 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2001-168572 [17]
AN 2001-07250 BIOTECHDS
AB An antisense compound (I) 8-30 bp in length targeted to a nucleic acid molecule encoding human SHP-2 (II), a member of the family of non-membrane protein-tyrosine-phosphatase (EC-3.1.3.48) in which (I) specifically hybridizees with and inhibits the expression of (II) is claimed. Also claimed is a composition containing (I) and a carrier or diluent. In an example, chimeric oligonucleotides were synthesized and cleaved from the support and the phosphate group was deprotected in 3:1 ammonia/ethanol at RT overnight then lyophilized. Treatment in methanolic ammonia for 14 hr was carried out to deprotect all bases. The pellet was resuspended for 24 hr to deprotect the 2' position. The oligonucleotide was recovered and analyzed for yield spectrophotometrically and for purity by capillary electrophoresis and mass spectrometry. The above can be used to produce (I) which is useful for inhibiting the expression of (II) in human cells or tissues. (I) is useful for treating a human with a disease associated with SHP-2 such as diabetes or an inflammatory disorder. The antisense compound can be used for diagnosis, therapeutics, prophylaxis. (98pp)

L7 ANSWER 78 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-05362 BIOTECHDS
TITLE: New chimeric nucleic acid vectors comprising adenoviral inverted terminal repeat flanking regions, and an internal region between the adenoviral flanking regions, useful for delivering therapeutic genes into a cell, or gene therapy;
inverted long terminal repeat chimeric adeno virus encoded in vector plasmid pDELTAE1S3PGK useful for e.g. adenosine-deaminase deficiency, sickle cell anemia, thalassemia, hemophilia-A, hemophilia-B, diabetes , tumor, braindisorder, Alzheimer disease, phenylketonuria, growth disorder, heart disease gene therapy and drug delivery
AUTHOR: AGUILAR-CORDOVA E
PATENT ASSIGNEE: BAYLOR COLLEGE MEDICINE
PATENT INFO: WO 2001091802 6 Dec 2001
APPLICATION INFO: WO 2000-US17453 30 May 2000
PRIORITY INFO: US 2000-207845 30 May 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-122041 [16]
AN 2002-05362 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Chimeric nucleic acid vectors comprising adenoviral inverted terminal repeat flanking regions, and an internal region between the adenoviral flanking regions, where the internal region contains retroviral long terminal repeat flanking regions flanking a cassette containing a nucleic acid region of interest, is new.
DETAILED DESCRIPTION - Chimeric nucleic acid vectors comprising adenoviral inverted terminal repeat flanking regions, and an internal region between the adenoviral flanking regions, where the internal region contains retroviral long terminal repeat flanking regions flanking a cassette containing a nucleic acid region of interest, is new. The chimeric nucleic acid vectors may additionally comprise: (a) a gag nucleic acid region between the adenoviral flanking regions; (b) a pol nucleic acid region between the adenoviral flanking regions; (c) a nucleic acid region between the adenoviral flanking regions selected from an env nucleic acid region, a nucleic acid region for pseudotyping a retroviral vector and a nucleic acid region for targeting a retroviral vector; (d) (a) and (b); (e) (a), (b) and (c); (f) (a), (b), (c) and a suicide nucleic acid region between the adenoviral flanking regions; (g) a rep nucleic acid region between the adenoviral flanking regions; (h) a cap nucleic acid region between the adenoviral flanking regions; (i) an adenoviral E4 nucleic acid region between the adenoviral

flanking regions; or (j) (f), (g) and (h). INDEPENDENT CLAIMS are also included for the following: (1) a chimeric plasmid, comprising: (a) adenoviral inverted terminal repeat flanking regions; (b) an internal region between the adenoviral flanking regions, where the internal region contains retroviral long terminal repeat flanking regions flanking a cassette containing a nucleic acid region of interest; (c) a gag nucleic acid region; (d) a pol nucleic acid region; and (e) a nucleic acid region between the adenoviral flanking regions selected from an env nucleic acid region, a nucleic acid region for pseudotyping a retroviral vector and a nucleic acid region for targeting a retroviral vector; and (
2) methods for producing retroviral virions.

BIOTECHNOLOGY - Preferred Vector: A chimeric nucleic acid vector as defined above which additionally comprise: (a) a nucleic acid region between the adenoviral flanking regions; (b) a gag and a pol nucleic acid region between the adenoviral flanking regions, and a nucleic acid region between the adenoviral flanking regions; or (c) a gag and a pol nucleic acid region between the adenoviral flanking regions, a nucleic acid region between the adenoviral flanking regions, and a suicide nucleic acid region between the adenoviral flanking regions, has a transactivator nucleic acid region located between the adenoviral flanking regions, and encodes a polypeptide which regulates the expression of the env nucleic acid. The transactivator is a tetracycline transactivator. The expression of the env nucleic acid region is regulated by an inducible promoter nucleic acid region. The inducible promoter nucleic acid region is induced by a stimulus selected from tetracycline, galactose, glucocorticoid, Ru487, and heat shock. The env nucleic acid region consists of an amphotropic envelope, xenotropic envelope, ecotropic envelope, human immunodeficiency virus 1 (HIV-1) envelope, human immunodeficiency virus 2 (HIV-2) envelope, feline immunodeficiency virus (FIV) envelope, simian immunodeficiency virus 1 (SIV) envelope, human T-cell leukemia virus 1 (HTLV-1) envelope, human T-cell leukemia virus 2 (HTLV-2) envelope or vesicular stomatitis virus-G glycoprotein. The suicide nucleic acid region is selected from Herpes simplex virus type 1 thymidine kinase, oxidoreductase, cytosine deaminase, thymidine kinase thymidilate kinase (Tdk::Tmk) and deoxycytidine kinase. Preferred Method: Producing retroviral virions comprises: (a) producing a chimeric nucleic acid vector comprising adenoviral inverted terminal repeat flanking regions, and an internal region between the adenoviral flanking regions, where the internal region contains retroviral long terminal repeat flanking regions flanking a cassette containing a nucleic acid region of interest; (b) introducing the chimeric nucleic acid vector to a cell which comprises a gag nucleic acid region, a pol nucleic acid region, an env nucleic acid region and a replication-defective helper vector consisting of E1 and E3 nucleic acid regions; and producing all infectious retroviral virion. Introduction of the chimeric nucleic acid vector occurs concomitantly.

Alternatively, the vector comprises adenoviral inverted terminal repeat flanking regions, an internal region between the adenoviral flanking regions, a gag nucleic acid region between the adenoviral flanking regions, a pol nucleic acid region between the adenoviral flanking regions, and a nucleic acid region between the adenoviral flanking regions. The method further comprises transducing the infectious retroviral virion to another cell, which is a hepatocytes, comprising a packaging region. The nucleic acid region of interest is selected from a reporter region, ras, myc, raf, erb, src, fms, jun, trk, ret, gsp, hst, bcl abl, Rb, CFTR, p16, p21, p27, p53, p57, p73, C-CAM, APC, CTS-1, zacl, scFV ras, DCC, NF-1, NF-2, WT-1, MEN-I, MEN-II, BRCA1, VHL, MMAC1, FCC, MCC, BRCA2, interleukin (IL)-l, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, granulocyte monocyte colony stimulating factor (GM-CSF), granulocyte (G)-CSF, thymidine kinase, CD40L, Factor VIII, Factor IX, CD40, multiple disease resistance (MDR), ornithine transcarbamylase (OTC), intracellular adhesion molecule (ICAM)-1, and insulin receptor.

ACTIVITY - Cytostatic; antidiabetic; antianemic; neuroprotective; nootropic; hemostatic; nootropic; dermatological. No biological data is given.

MECHANISM OF ACTION - Gene therapy.

USE - The vectors are used for the delivery and stable integration of therapeutic constructs and eliminate some of the limitations currently encountered with in vivo gene transfer applications. The vectors are especially useful in gene therapy. Cells infected with the chimeric vectors can be used to treat a variety of diseases including adenosine deaminase deficiency, sickle cell anemia, thalassemia, hemophilia A or B, diabetes, cancer, brain disorders such as Alzheimer's disease, phenylketonuria and other illnesses such as growth disorders and heart diseases.

ADMINISTRATION - The vectors can be administered to the cell by physical means such as electroporation, gene gun or application of large volumes of a liquid (pressure), or by complexing the vector to another entity such as liposome or transporter molecule.

ADVANTAGE - The chimeric vector overcomes the problem of short in vivo retroviral half-lives. The chimeric delta-adeno/retroviral vector is designed to have the ability to be grown to very high titers with the added advantage of additionally generating high titer retroviral vectors in vigor and in vivo. The vector allows all cis and trans components of retroviral vector to be incorporated as multiple transcriptional units into one vector, and this vector design overcomes some of the cytotoxicity limitations of earlier generation of adenoviral vectors that express late viral gene products. The new vectors does not necessarily increase the risks presently associated with either retroviral or adenoviral vectors, however, it allows the exploitation of the in vivo infectivity of adenoviruses and the long-term expression from

retroviruses.

EXAMPLE - The minimal amphoteric Moloney murine leukemia virus backbone (S3) and the cassette for the phosphoglycerate kinase (PGK) promoter driving neomycin fused in-frame to the 3' end of the lacZ gene (pPGK-betageobpA) was subcloned into pDELTAE1sp1B. S3 was cut with Eco RI and ScaI, and cloned into pDELTAE1sp1B. The resulting plasmid was designated pDELTAE1S3. PGKbetageobp.A was directionally sub-cloned into S3 of pDELTAE1S3 forming pDELTAE1S3PGK. Restriction enzyme digests followed by electrophoresis analysis confirmed the orientation and identification of full-length inserts from each subclone.(114 pages)

L7 ANSWER 79 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-05428 BIOTECHDS
TITLE: Novel 1405 isolated polypeptides, useful for diagnosis, treatment and prevention of neural, immune system, muscular, reproductive, gastrointestinal, pulmonary, cardiovascular, renal and proliferative disorders;
involving vector-mediated gene transfer for expression in host cell, for use in drug screening, disease diagnosis, prevention, therapy, gene therapy and DNA chips
AUTHOR: BIRSE C E; ROSEN C A
PATENT ASSIGNEE: HUMAN GENOME SCI INC
PATENT INFO: WO 2001090304 29 Nov 2001
APPLICATION INFO: WO 2000-US16450 19 May 2000
PRIORITY INFO: US 2000-205515 19 May 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-122018 [16]
AN 2002-05428 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - One thousand, four hundred and five polypeptide sequences not defined in the specification, are new.

DETAILED DESCRIPTION - One thousand, four hundred and five polypeptide sequences (S1) not defined in the specification, are new. An isolated polypeptide (I) selected from S1 comprises a sequence at least 90% identical to a sequence selected from: (a) a polypeptide fragment of a sequence selected from (S1) or encoded sequence contained in cDNA clone ID Number (C) given in specification; (b) a polypeptide fragment having biological activity, a polypeptide domain, epitope or full length protein of a sequence selected from S1 or encoded sequence contained in (C); or (c) a variant, allelic variant or species homolog of a sequence selected from S1. INDEPENDENT CLAIMS are also included for the following: (1) an isolated nucleic acid molecule (II) comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from: (a) a polynucleotide fragment of a sequence selected from one of the 1405

nucleotide sequence (S2) not defined in the specification or a polynucleotide fragment of the cDNA sequence contained in (C), which is hybridizable to a sequence selected from S2; (b) a polynucleotide encoding a polypeptide fragment of a sequence selected from S1 or a polypeptide fragment encoded by the cDNA sequence contained in (C), which is hybridizable to a sequence selected from S2; (c) a polynucleotide encoding a polypeptide fragment of a polypeptide encoded by a sequence selected from S2 or a polypeptide fragment encoded by the cDNA sequence contained in (C), which is hybridizable to a sequence selected from S2; (d) a polynucleotide encoding a polypeptide domain or polypeptide epitope of a sequence selected from S1 or a polypeptide domain or epitope encoded by the cDNA sequence contained in (C), which is hybridizable to a sequence selected from S2; (e) a polynucleotide encoding a polypeptide selected from S1 or the cDNA sequence contained in (C), which is hybridizable to a sequence selected from S2, having biological activity; (f) a polynucleotide which is a variant or an allelic variant of a sequence selected from S2; (g) a polynucleotide which encodes a species homolog of a sequence selected from S1; and (h) a polynucleotide capable of hybridizing under stringent conditions to the above mentioned polynucleotides, where the polynucleotide does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide sequence of only A residues or of only T residues; (2) a recombinant vector (III) comprising (II); (3) a method of making a recombinant host cell comprising (II); (4) a recombinant host cell (IV) produced by the above mentioned method; (5) an isolated antibody (V) that binds specifically to (I); (6) a recombinant host cell (VI) that expresses (I); (7) production of (I); (8) a polypeptide produced by the above mentioned method; (9) identifying (M1) a binding partner to (I) involves contacting (I) with a binding partner, and determining whether the binding partner effects an activity of (I); (10) a product produced by M1; (11) a gene corresponding to the cDNA sequence of a sequence selected from S1; (12) identifying (M2) an activity in a biological assay, involves expressing a sequence selected from S2 in a cell, isolating the supernatant, detecting an activity in a biological assay, and identifying the protein in the supernatant in the activity; and (13) diagnosing (M3) a pathological condition or a susceptibility to a pathological condition in a subject.

WIDER DISCLOSURE - Also disclosed are: (1) a screening method for identifying agonists and antagonists of (I) or (II); (2) a method and/or composition for inhibiting or enhancing the production and function of (I); (3) diagnostic and therapeutic methods for diagnosing, treating, preventing and/or prognosing disorders related to (I); (4) a polypeptide having one or more residues deleted from the amino terminus or carboxy terminus of S1; (5) a polynucleotide encoding fusion proteins; (6) chemically modified derivatives of (I); (7) an antibody that competitively inhibits binding of an antibody to an epitope of (I); (8) a

method of generating monoclonal antibodies; (9) an antibody produced by the above mentioned method; (10) a method for generating polyclonal or monoclonal human antibodies against (I); (11) a polynucleotide comprising a sequence encoding (V) or its fragments; (12) a composition comprising (I) fused or conjugated to antibody domains other than the variable regions; (13) an antibody or its fragments conjugated to a diagnostic or therapeutic agent; and (14) a kit for analyzing samples for the presence of proliferative and/or cancerous polynucleotides derived from a test subject, comprising a polynucleotide probe contained in the nucleotide sequence that specifically hybridizes to (II) and a suitable container.

BIOTECHNOLOGY - Preparation: (I) is produced by culturing (VI) under conditions such that (I) is expressed and recovering (I) (claimed). Preferred Polypeptide: In (I), the full length protein comprises sequential amino acid deletions, from either the C-terminus or the N-terminus. Preferred Polynucleotide: In (II), the polynucleotide fragment comprises a nucleotide sequence encoding a protein. The polynucleotide fragment comprises a nucleotide sequence encoding a sequence selected from S1 or the polypeptide encoded by the cDNA sequence contained in (C), which is hybridizable to a sequence selected from S2. The polynucleotide fragment comprises the entire nucleotide sequence of a sequence selected from S2 or the cDNA sequence contained in (C), which is hybridizable to a sequence selected from S2. (I) comprises sequential nucleotide deletion from either the C-terminus or the N-terminus. Preferred Host Cell: (IV) comprises vector sequences. Preferred Method: M3 comprises: (a) determining the presence or absence of a mutation in (II), and diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or absence of the mutation; or (b) determining the presence or amount of expression of (I) in a biological sample, and diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or amount of expression of (I).

ACTIVITY - Cytostatic; dermatological; immunosuppressive; antiinflammatory; antirheumatic; antiarthritic; antithyroid; thyromimetic; nephrotropic; antiallergic; antiasthmatic; neuroprotective; antiparkinsonian; anti-HIV; nootropic; antiarteriosclerotic; anticoagulant; thrombolytic; vulnerary; antileprotic.

MECHANISM OF ACTION - Gene therapy. No supporting biological data is given.

USE - (I) or (II) is useful for preventing, treating, or ameliorating a medical condition, by administering to a mammalian subject a therapeutically effective amount of (I) or (II).(claimed). (I), (II) or (V) is useful for diagnosis, treatment and prevention of diseases associated with cell proliferation and cell signaling, particularly cancer, immune response and neuronal disorders, to treat or prevent neural disorders, immune system disorders, muscular disorder, reproductive disorders, gastrointestinal disorders, pulmonary disorders,

cardiovascular disorders, renal disorders, proliferative disorders, and/or cancerous diseases and conditions. (I), (II) or (V) is useful for treating autoimmune disorders such as systemic lupus erythematosus, rheumatoid arthritis, sclerosis, autoimmune thyroiditis, Hashimoto's thyroiditis, Goodpasture's syndrome, Grave's disease, insulin-resistant diabetes mellitus, as immuno suppressive agents, for treating allergic reactions and conditions such as asthma, for treating inflammatory conditions such as Crohn's disease, respiratory disorders such as allergy, gastrointestinal disorders such as inflammatory bowel disease, cancer, central nervous system disease such as multiple sclerosis, neurodegenerative disorders such as Parkinson's disease, AIDS-related dementia, cardiovascular disease such as atherosclerosis, graft-versus-host disease, blood-related disorders such as thrombosis, hyperproliferative disorders, renal disorders such as glomerulonephritis, for birth control, for wound healing and epithelial cell proliferation, and for treating infectious diseases such as leprosy. (I) or (V) is useful to provide immunological probes for differential identification of tissues or cell types, as molecular weight markers on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels or on molecular sieve gel filtration columns, to raise antibodies and to test biological activities. (II) is useful in gene therapy, for chromosome identification, for radiation hybrid mapping, HAPPY mapping, and long range restriction mapping, for identifying individuals from minute biological samples, as an alternative to restriction fragment length polymorphism (RFLP), as polymorphic markers for forensic purposes, as a hybridization probe for differential identification of the tissue(s) or cell type(s) present in a biological sample, as molecular weight markers on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, as a probe to subtract-out known sequences in the process of discovering novel polynucleotides, for selecting and making oligomers for attachment to a gene chip or other support, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response.

ADMINISTRATION - (I) or (V) is administered by intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, oral, intraventricular or intrathecal route. (V) is administered at a dose of 0.1-100 mg/kg, preferably 1-10 mg/kg. (II) is administered by systemic, intravenous, oral, or percutaneous route at a dose of 0.05-50 mg/kg, preferably 0.05-5 mg/kg.

EXAMPLE - Isolating a particular clone from the deposited sample of plasmid cDNAs in ATCC deposit No. PTA-1559 was carried out as follows. First, a plasmid was directly isolated by screening the clones using a polynucleotide probe corresponding to the nucleotide sequence, X as defined in specification. Particularly, a specific polynucleotide with

30-40 nucleotides in synthesized. The oligonucleotide was labeled with 32P-gamma-ATP using T4 polynucleotide kinase and purified. The plasmid mixture was transformed into a suitable host. The transformants were plated on 1.5% agar plates (containing the appropriate selection agent, e.g., ampicillin) to a density of about 150 transformants (colonies) per plate. These plates were screened using Nylon membranes. Alternatively, two primers of 17-20 nucleotides derived from both ends of the nucleotide sequence X were synthesized and used to amplify the desired cDNA using the deposited cDNA plasmid as a template. The polymerase chain reaction (PCR) was carried out under routine conditions. The amplified product was analyzed by agarose gel electrophoresis and the DNA bound with expected molecular weight was excised and purified. The PCR product was verified to be the selected sequence by subcloning and sequencing the DNA product.(2081 pages)

L7 ANSWER 80 OF 150 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 2002-08252 BIOTECHDS
TITLE: Preparing A liquid sample for fractionation, useful in analysis of serum for marker proteins, by removing abundant proteins, especially serum albumin and immunoglobulins;
mouse hybridoma cell culture for monoclonal antibody production for osteoporosis, arthritis, cancer, cardiovascular disease and Paget disease diagnosis and therapy
AUTHOR: STEVENS A C
PATENT ASSIGNEE: FISH and RICHARDSON P C
PATENT INFO: US 2001051380 13 Dec 2001
APPLICATION INFO: US 1998-17284 2 Feb 1998
PRIORITY INFO: US 1998-17284 2 Feb 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-097072 [13]
AN 2002-08252 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Preparing a liquid sample (A) for fractionation by treating (A) with a polypeptide affinity reagent (I) having specific binding affinity for an abundant macromolecule (II) in the sample, to form a (I)-(II) complex. (I) is one member of a high-affinity binding pair and the complex is then treated with the other component of this pair to form a collapsible affinity matrix.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the
following: (a) serum substantially and specifically depleted in serum albumin (SA) and immunoglobulins (Ig); (b) monoclonal antibody (MAb) that

can immunoprecipitate SA from serum; (c) host cells that express the MAb HSA2126NX.012; and (d) kit comprising, in separate containers, MAb bound to one member of a high-affinity binding pair and the other member of the binding pair.

USE - The method is especially used to remove serum albumin and immunoglobulins from serum, particularly so that minor serum proteins (particularly new diagnostic or therapeutic markers of e.g. osteoporosis, arthritis, cancer, cardiovascular diseases and Paget's disease) can be detected, especially by two -dimensional gel electrophoresis.

ADVANTAGE - Removal of (II) facilitates fractionation of other serum proteins that would otherwise be obscured, allowing many of them to be detected relatively rapidly. The collapsible nature of the matrix means that less protein is trapped in void spaces. Human serum was treated with biotinylated antibody HSA2126NX.012 (specific for human serum albumin) and avidin, also with gamma-binding protein A to deplete immunoglobulins. The treated sample was then subjected to two-dimensional polyacrylamide gel electrophoresis. Compared with a sample treated with immobilized Cibacron blue for removal of albumin, the present method produced 20% more spots and did not result in loss of the serum glycoprotein HC gp39. (24 pages)

L7 ANSWER 81 OF 150 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:833374 CAPLUS
DOCUMENT NUMBER: 136:1661
TITLE: Nucleic acids and polypeptides for 33 human ion channels and uses thereof
INVENTOR(S): Roberds, Steven L.; Benjamin, Christopher W.; Karnovsky, Alla M.; Ruble, Cara L.
PATENT ASSIGNEE(S): Pharmacia & Upjohn Company, USA
SOURCE: PCT Int. Appl., 172 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001085788	A2	20011115	WO 2001-US14965	20010510
WO 2001085788	A3	20020718		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US,

UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
EP 1280824 A2 20030205 EP 2001-931099 20010510
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR
PRIORITY APPLN. INFO.: US 2000-203305P P 20000510
US 2000-206526P P 20000523
US 2000-207033P P 20000525
US 2000-207092P P 20000525
US 2000-207093P P 20000525
US 2000-216893P P 20000707
US 2000-223245P P 20000804
US 2000-237873P P 20001004
WO 2001-US14965 W 20010510

AB The present invention provides novel ion channel polypeptides and polynucleotides that identify and encode them. In addn., the invention provides expression vectors, host cells and methods for their prodn. The invention also provides methods for the identification of ion channel agonists/antagonists, useful for the treatment of human diseases and conditions. Use of the ion channel polypeptides and polynucleotides for diagnosis and treatment of brain and mental disorders is claimed. Nucleic acids and polypeptides for 33 human ion channels, numbered 15-30, 96-102, and 119-128, were identified as sequence homologs of known ion channels by searching databases using query sequences from the SWISSPROT database. MRNA for ion channel 15 was detected in brain, fetal brain, kidney, lung, muscle, testis, heart, liver, small intestine, spleen, and peripheral blood leukocytes. Ion channel 17 mRNA was detected in retina, brain, fetal brain, kidney, and testis and ion channel 19 mRNA was detected in fetal brain, heart, and muscle. The mRNA expression profiles suggest that these channels could be a target for treatment of neurol. and psychiatric disorders, cardiomyopathies or arrhythmias, lung diseases, inflammation, smooth muscle proliferation, spasm, eye diseases, hypertension, and reproductive disorders.

L7 ANSWER 82 OF 150 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:798478 CAPLUS
DOCUMENT NUMBER: 135:340279
TITLE: A novel G protein-coupled receptor sequence homolog 4941, and related methods and compositions for the diagnosis and treatment of cardiovascular and tumorigenic disease
INVENTOR(S): Galvin, Katherine A.; Rudolph-Owen, Laura A.
PATENT ASSIGNEE(S): Millennium Pharmaceuticals, Inc., USA
SOURCE: PCT Int. Appl., 118 pp.

CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001081634	A2	20011101	WO 2001-US13788	20010425

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

EP 1280937	A2	20030205	EP 2001-930917	20010425

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR

PRIORITY APPLN. INFO.: US 2000-199908P P 20000426
US 2000-635521 A 20000809
WO 2001-US13788 W 20010425

AB The invention provides protein and cDNA sequences for a novel human G protein-coupled receptor (GPCR) sequence homolog 4941. GPCR 4941 gene is located at human chromosome 2q21-22 and its mRNA tissue profile is provided. Specifically, the present invention identifies GPCR 4941 genes which are differentially expressed in cardiovascular disease states, relative to their expression in normal, or non-cardiovascular disease states, and/or in response to manipulations relevant to cardiovascular disease. The present invention also identifies GPCR 4941 genes as differentially expressed in tumorigenic disease , e.g., ovarian cancer. The present invention relates to methods and compns. for the diagnosis and treatment of cardiovascular disease and cancers. These diseases include but not limit, atherosclerosis reperfusion injury, hypertension, restenosis, arterial inflammation, and endothelial cell disorders, such as disorders assocd. with aberrant endothelial cell growth, angiogenesis and/or vascularization, e.g., tumorigenic disorders. The present invention describes methods for the diagnostic evaluation and prognosis of various cardiovascular and tumorigenic diseases, and for the identification of subjects exhibiting a predisposition to such conditions. The present invention provides methods for the diagnostic monitoring of patients undergoing clin. evaluation for the treatment of cardiovascular disease and tumorigenic, and for monitoring the efficacy of

compds. in clin. trials. The present invention also provides methods for the identification and therapeutic use of compds. as treatments of cardiovascular and tumorigenic disease.

L7 ANSWER 83 OF 150 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:208458 CAPLUS
DOCUMENT NUMBER: 134:251186
TITLE: Methods of using a major histocompatibility complex class III haplotype to diagnose autoimmune disease, especially Crohn's disease
INVENTOR(S): Taylor, Kent D.; Rotter, Jerome I.; Yang, Huiying
PATENT ASSIGNEE(S): Cedars-Sinai Medical Center, USA
SOURCE: PCT Int. Appl., 56 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001020036	A2	20010322	WO 2000-US25112	20000913
WO 2001020036	A3	20011206		
W: AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 6376176	B1	20020423	US 1999-395345	19990913
EP 1214451	A2	20020619	EP 2000-961884	20000913
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL				
US 2002150939	A1	20021017	US 2002-75425	20020212

PRIORITY APPLN. INFO.: US 1999-395345 A2 19990913
WO 2000-US25112 W 20000913

AB The present invention provides a method of diagnosing or predicting susceptibility to an autoimmune disease in an individual by detg. the presence or absence in the individual of a 2-2 -4 haplotype at the Notch4, HSP70-HOM and D6S273 loci, where the presence of the haplotype diagnoses or predicts susceptibility to the autoimmune disease. The methods of the invention can be particularly useful

for diagnosing or predicting susceptibility to Crohn's disease, rheumatoid arthritis or type I diabetes mellitus. In a preferred embodiment, a method of the invention is used to diagnose or predict susceptibility to Crohn's disease in an individual of Ashkenazi Jewish ethnicity.

L7 ANSWER 84 OF 150 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 2001665387 MEDLINE
DOCUMENT NUMBER: 21566953 PubMed ID: 11710714
TITLE: Preferential recognition of a fragment species of osteoarthritic synovial fluid fibronectin by antibodies to the alternatively spliced EIIIA segment.
AUTHOR: Peters J H; Carsons S; Kalunian K; McDougall S; Yoshida M; Ko F; van der Vliet-Hristova M; Hahn T J
CORPORATE SOURCE: West Los Angeles VA Medical Center, and UCLA School of Medicine, California, USA.. John.Peters3@med.va.gov
CONTRACT NUMBER: AR-42469 (NIAMS)
P60-AG-10415 (NIA)
SOURCE: ARTHRITIS AND RHEUMATISM, (2001 Nov) 44 (11) 2572-85.
Journal code: 0370605. ISSN: 0004-3591.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200112
ENTRY DATE: Entered STN: 20011119
Last Updated on STN: 20020123
Entered Medline: 20011205
AB OBJECTIVE: To characterize the species of synovial fluid (SF) fibronectin (FN) bearing the alternatively spliced EIIIA segment. METHODS: SF from patients with osteoarthritis (OA) and rheumatoid arthritis (RA), as well as corresponding affinity isolation products, were subjected to 1-dimensional and 2-dimensional electrophoresis followed by Western blot analysis. RESULTS: Regardless of the clinical type of arthritis, a polyclonal antibody that recognizes antigenic determinants throughout the FN molecule produced staining of predominantly approximately 200+ and approximately 170-kd species in reduced 1-dimensional electrophoresis. Despite the overall prevalence of the larger species, 4 monoclonal antibodies (mAb) reactive with sequences lying near the center of the EIIIA segment exhibited a relative failure to recognize the larger of these 2 species in OA, but not RA, SF. The absence of recognition of EIIIA sequences within the approximately 200+ kd forms of OA SF FN was unrelated to their derivation from dimers, since anti-EIIIA mAb recognized the smaller fragment species in preference to both monomeric and dimeric forms. The approximately 170-kd EIIIA+ fragments were observed to have minimal gelatin-binding capacity and

appeared on 2-dimensional electrophoresis to extend from the N-terminus of FN through at least the center of the EIIIA segment. Similar results were obtained for samples obtained by needle aspiration or arthroscopic lavage, suggesting a widespread applicability of these findings. CONCLUSION: The approximately 170-kd EIIIA+ species of FN could potentially constitute a soluble "vehicle" by which chondrocyte-regulating EIIIA sequences, liberated from inhibitory flanking C-terminal sequences, could reach cells in the arthritic joint. Additionally, "FN species-specific" recognition of this segment within OA SF could constitute a marker by which to gauge the activity of the OA disease process.

L7 ANSWER 85 OF 150 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:18064 CAPLUS
DOCUMENT NUMBER: 136:214681
TITLE: Reduced HDL particle size as an additional feature of the atherogenic dyslipidemia of abdominal obesity
AUTHOR(S): Pascot, Agnes; Lemieux, Isabelle; Prud'homme, Denis; Tremblay, Angelo; Nadeau, Andre; Couillard, Charles; Bergeron, Jean; Lamarche, Benoit; Despres, Jean-Pierre
CORPORATE SOURCE: Lipid Research Center, CHUL Research Center, Ste-Foy, QC, G1V 4G5, Can.
SOURCE: Journal of Lipid Research (2001), 42(12), 2007-2014
CODEN: JLPRAW; ISSN: 0022-2275
PUBLISHER: Lipid Research, Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Reduced plasma HDL cholesterol concn. has been assocd. with an increased risk of coronary heart disease. However, a low HDL cholesterol concn. is usually not obsd. as an isolated disorder because this condition is often accompanied by addnl. metabolic alterations. The objective of this study was to document the relevance of assessing HDL particle size as another feature of the atherogenic dyslipidemia found among subjects with visceral obesity and insulin resistance. For that purpose, an av. HDL particle size was computed by calcg. an integrated HDL particle size using nondenaturing 4-30% gradient gel electrophoresis. Potential assocns. between this av. HDL particle size vs. morphometric and metabolic features of visceral obesity were examd. in a sample of 238 men. Results of this study indicated that HDL particle size was a significant correlate of several features of an atherogenic dyslipidemic profile such as increased plasma TG, decreased HDL cholesterol, high apolipoprotein B, elevated cholesterol/HDL cholesterol ratio, and small LDL particles as well as increased levels of visceral adipose tissue (AT) (0.33 .ltoreq. r .ltoreq. 0.61, $P < 0.0001$). Thus, men with large HDL particles had a more favorable plasma lipoprotein-lipid profile compared

with those with smaller HDL particles. Furthermore, men with large HDL particles were also characterized by reduced overall adiposity and lower levels of visceral AT as well as reduced insulinemic-glycemic responses to an oral glucose load. In conclusion, small HDL particle size appears to represent another feature of the high TG-low HDL cholesterol dyslipidemia found in viscerally obese subjects characterized by hyperinsulinemia.
REFERENCE COUNT: 50 THERE ARE 50 CITED REFERENCES AVAILABLE FOR THIS
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 86 OF 150 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2001:345282 BIOSIS
DOCUMENT NUMBER: PREV200100345282
TITLE: Actin and annexins I and II are among the main endothelial plasmalemma-associated proteins forming early glucose adducts in experimental diabetes.
AUTHOR(S): Ghitescu, Lucian D. (1); Gugliucci, Alejandro; Dumas, France
CORPORATE SOURCE: (1) Departement de Pathologie et Biologie Cellulaire, Universite de Montreal, Succursale Centre-ville, Montreal, PQ, H3C 3J7: ghitescd@patho.umontreal.ca Canada
SOURCE: Diabetes, (July, 2001) Vol. 50, No. 7, pp. 1666-1674. print.
ISSN: 0012-1797.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB An immunochemical and biochemical study was performed to reveal which of the endothelial plasma membrane proteins become glycated during the early phases of diabetes. The blood front of the lung microvascular endothelial plasmalemma was purified by the cationic colloidal silica method from normal and diabetic (streptozotocin-induced) rats and comparatively analyzed by two-dimensional electrophoresis. No major qualitative differences in the general spectrum of endothelial plasmalemmal proteins were recorded between normoglycemic and hyperglycemic animals. By probing with anti-glucitollysine antibodies, we found that at 1 month after the onset of diabetes, several endothelial membrane polypeptides contained glucose covalently linked to their lysyl residues. Ten days of insulin treatment restored euglycemia in the diabetic animals and completely abolished the membrane nonenzymatic glycosylation. All the glycated polypeptides of the endothelial plasma membrane belong to the peripheral type and are associated with its cytoplasmic face (cell cortex). They were solubilized by buffers of high pH and were not detected in the lung cytosolic fraction (100,000 g). By microsequencing, the major proteins

labeled by the anti-glucitollysine have been identified as being actin, annexin I, annexin II, the p34 subunit of the Arp2/3 complex, and the Ras suppressor protein-1. Conversely, the intrinsic endothelial membrane proteins do not seem to be affected by hyperglycemia. This defines the internal face of the endothelial plasma membrane, particularly the cortical cytoskeleton, as a preferential target for nonenzymatic glycosylation in diabetes, with possible consequences on the fluidity of the endothelial plasmalemma and impairment of the endothelial mechano-transducing ability.

L7 ANSWER 87 OF 150 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

DUPLICATE 6

ACCESSION NUMBER: 2001:495668 BIOSIS

DOCUMENT NUMBER: PREV200100495668

TITLE: Dinucleotide repeat polymorphism of matrix metalloproteinase-9 gene is associated with diabetic nephropathy.

AUTHOR(S): Maeda, Shiro; Haneda, Masakazu (1); Guo, Baoliang; Koya, Daisuke; Hayashi, Kazuyuki; Sugimoto, Toshiro; Isshiki, Keiji; Yasuda, Hitoshi; Kashiwagi, Atsunori; Kikkawa, Ryuichi

CORPORATE SOURCE: (1) Third Department of Medicine, Shiga University of Medical Science, Seta, Otsu, Shiga, 520-2192: haneda@belle.shiga-med.ac.jp Japan

SOURCE: Kidney International, (October, 2001) Vol. 60, No. 4, pp. 1428-1434. print.
ISSN: 0085-2538.

DOCUMENT TYPE: Article

LANGUAGE: English

SUMMARY LANGUAGE: English

AB Background: Although genetic susceptibility has been proposed as an important factor for the development and progression of diabetic nephropathy, the definitive gene has not been identified. To identify the genetic marker for diabetic nephropathy, we examined the association between the (A-C)n dinucleotide repeat polymorphism upstream of the matrix metalloproteinase-9 (MMP-9) gene and diabetic nephropathy in a group of Japanese patients with type 2 diabetes.
Methods: Patients were divided into three groups based on their urinary albumin excretion rate (AER) and the stage of diabetic retinopathy as follows: uncomplicated group (U), normal albuminuria (AER<20 mug/min) without proliferative retinopathy and with the duration of diabetes more than 20 years (N=32); microalbuminuria group (M), 20ltoreqAER<200 mug/min (N=155); overt nephropathy group (O), AERgtoreq200 mug/min (N=63). The region containing the dinucleotide repeat upstream of MMP-9 gene was amplified by polymerase chain reaction (PCR). The amplified

products were analyzed with 7% formamide/urea acrylamide gel electrophoresis. The promoter constructs of the MMP-9 gene were transfected with the CMV-beta-galactosidase construct into 293 cells using the liposome method. Twenty-four hours after transfection, cells were harvested, and luciferase and beta-galactosidase activities were measured. Results: Nine alleles of the dinucleotide repeat polymorphism (17 to 25 repeats) were identified, and the frequency of each allele in diabetic subjects was not different from that in nondiabetic controls. The frequency of the allele containing 21 repeats (A21) was most abundant (42.4% in control and 45.6% in diabetic subjects), followed by the allele with 23 repeats (A23; 35.4% in control and 27.6% in diabetic subjects). The A21 allele was less frequent in M and O than U (O, 38.9%; M, 45.5%; U, 59.3%, chi2=7.18; P<0.05, O vs. U), while the frequency of the alleles other than A21 was not different among each group. The calculated odds ratio for nephropathy in the noncarrier, heterozygote, or homozygote of A21 allele was 3.38, 1.97, and 0.2, respectively. Furthermore, the promoter assay for the MMP-9 gene revealed that the A21 allele had a higher promoter activity compared with other alleles. No significant correlation was observed between serum MMP-9 concentrations and the MMP-9 gene polymorphism. Conclusion: These results indicate that the patients with A21 allele of the MMP-9 gene may be protected from the development and progression of diabetic nephropathy. Thus, the micro-satellite polymorphism upstream of the MMP-9 gene could be a useful genetic marker for diabetic nephropathy.

L7 ANSWER 88 OF 150 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
DUPLICATE 7
ACCESSION NUMBER: 2002:100961 BIOSIS
DOCUMENT NUMBER: PREV200200100961
TITLE: The association between PPP1R3 gene polymorphisms and type 2 diabetes mellitus.
AUTHOR(S): Wang Guoying (1); Qian Rongli; Li Qiongfang; Niu Tianhua; Chen Changzhong; Xu Xiping
CORPORATE SOURCE: (1) Department of Endocrinology, Third Affiliated Hospital of Beijing University, Beijing, 100083: wanggy88@hotmail.com China
SOURCE: Chinese Medical Journal (English Edition), (December, 2001) Vol. 114, No. 12, pp. 1258-1262. print.
ISSN: 0366-6999.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Objective: To detect the relationship between the polymorphism of the glycogen-targeting regulatory subunit of the skeletal muscle glycogen-associated protein phosphatase 1 (PPP1R3) gene and type 2 diabetes by case-control study. Methods: We genotyped the PPP1R3

gene Asp905Tyr polymorphism and a common 3'-untranslated region AT (AU)-rich element (ARE) polymorphism in 101 type 2 diabetic patients and 101 controls by oligonucleotide ligation assay (OLA) and polyacrylamide gel elecrophoresis, respectively. Results: Subjects with Tyr/Tyr genotypes whose body mass index (BMI) <25 were used as the reference group. Those whose BMIgtoreq25 with Asp905 had a 3.66-fold increase (95% CI: 1.48-9.06, P=0.005) in type 2 diabetes risk. No association was found between 3'UTR ARE polymorphism and type 2 diabetes mellitus (OR=1.15; 95% CI: 0.62-2 .14, P=0.65). Conclusion: A joint effect between the Asp905 and BMI increases the risk of type 2 diabetes, and Asp905Tyr and ARE polymorphism of PPP1R3 gene are not the major diabetogenic gene variants in Chinese population.

L7 ANSWER 89 OF 150 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:74727 CAPLUS
DOCUMENT NUMBER: 137:120104
TITLE: From genome to proteome
AUTHOR(S): Grus, F. H.; Augustin, A. J.; Pfeiffer, N.; Schmidt-Erfurth, U.
CORPORATE SOURCE: Universitats-Augenklinik, Mainz, 55101, Germany
SOURCE: Ophthalmologe (2001), 98(12), 1132-1137
CODEN: OHTHEJ; ISSN: 0941-293X
PUBLISHER: Springer-Verlag
DOCUMENT TYPE: Journal; General Review
LANGUAGE: German
AB A review on application of DNA microarrays in simultaneous investigation of diverse disease-assocd. gene expressions. Proteome anal., 2-dimensional gel electrophoresis of tear protein patterns of diabetic patients and patients with Sicca syndrome, and data evaluation by bioinformatics using neuronal networks are described.
REFERENCE COUNT: 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 90 OF 150 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 2001413726 MEDLINE
DOCUMENT NUMBER: 21356771 PubMed ID: 11464256
TITLE: Systematic screening of type B human natriuretic peptide receptor gene polymorphisms and association with essential hypertension.
AUTHOR: Rahmutula D; Nakayama T; Soma M; Sato M; Izumi Y; Kanmatsuse K; Ozawa Y
CORPORATE SOURCE: The Second Department of Internal Medicine, Nihon University School of Medicine, Tokyo, Japan.
SOURCE: JOURNAL OF HUMAN HYPERTENSION, (2001 Jul) 15 (7) 471-4.

Journal code: 8811625. ISSN: 0950-9240.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200110
ENTRY DATE: Entered STN: 20011015
Last Updated on STN: 20011015
Entered Medline: 20011011

AB C-type natriuretic peptide (CNP) dilates arteries, lowers blood pressure and inhibits proliferation of vascular smooth muscle cells via the type B natriuretic peptide receptor (NPRB). The CNP-NPRB system may play a crucial role in the development of cardiovascular disease. We recently determined the structure of the human NPRB gene. In the present study, our objectives are to identify the polymorphisms of the NPRB gene and investigate the association of this gene with essential hypertension (EH). We used the polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) technique to study the NPRB gene polymorphism, and conducted an association study using a novel polymorphic marker. PCR-SSCP analysis of all 22 exons was done in 90 subjects, and abnormally-migrating bands were observed in the analyses of exon 11 and intron 18. Direct sequencing of these DNA fragments revealed the following sequence alterations: a C to T transition at nucleotide (nt) 2077 in exon 11 and a 9-bp insertion/deletion (I/D) in intron 18. PCR-restriction fragment length polymorphism analysis (PCR-RFLP) was developed to detect the C2077T transition. PCR-RFLP analyses of healthy subjects revealed that the C2077T polymorphism had complete linkage to GT repeats in intron 2 reported previously. The I/D polymorphism was identified by polyacrylamide gel electrophoresis, and it was not linked to any known polymorphic alleles of this gene. Therefore, the possible association between the I/D polymorphism and EH was investigated. A total of 123 individuals with EH and 123 age-matched normotensive control subjects were studied. Overall distributions of allele frequencies in the two groups were not significantly different. Although the I/D polymorphism in intron 18 of the NPRB gene was not associated with EH, the results of this study, which identified two novel polymorphisms in the human NPRB gene, will facilitate further genetic analysis of this gene and cardiovascular disease.

L7 ANSWER 91 OF 150 MEDLINE
ACCESSION NUMBER: 2001672158 MEDLINE
DOCUMENT NUMBER: 21575399 PubMed ID: 11718066
TITLE: Multivariate logistic regression analysis on the risk factors of type 2 diabetes mellitus.
AUTHOR: Lu Y; Miao H; Wang H

CORPORATE SOURCE: Department of Endocriology, Second Affiliated Hospital of Nanjing Medical University, Nanjing 210011, China.
SOURCE: CHUNG-HUA LIU HSING PING HSUEH TSA CHIH CHINESE JOURNAL OF
EPIDEMIOLOGY, (2001 Aug) 22 (4) 277-80.
Journal code: 8208604. ISSN: 0254-6450.
PUB. COUNTRY: China
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: Chinese
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200112
ENTRY DATE: Entered STN: 20011126
Last Updated on STN: 20020123
Entered Medline: 20011205

AB OBJECTIVE: To explore the relationship between multivariate factors and type 2 diabetes mellitus. METHODS: Polymorphisms of microsatellite markers in uncoupling protein 3 (UCP3) gene, hormone-sensitive lipase (HSL) gene and protein tyrosine phosphatase-1B (PTP-1B) gene were tested in 106 patients with type 2 diabetes and 102 control subjects by performing polymerase chain reaction (PCR), polyacrylamide gel electrophoresis and silver staining. Multivariate logistic regression analysis was performed by all factors. RESULTS: Through univariate analysis, type 2 diabetes had significantly positive associations with age, systolic blood pressure (SBP), fasting insulin (FINS) level, cholesterol, triglyceride, low-density lipoprotein, apolipoprotein B (ApoB), alpha lipoprotein, UCP3 gene allele 6, UCP3 gene allele 7, HSL gene allele 9, and negative associations with UCP3 gene allele 1 and 3, HSL gene allele 5, high-density lipoprotein. The results of multivariate logistic analysis showed that UCP3 gene allele 6, UCP3 gene allele 7, SBP, ApoB, alpha lipoprotein were still positively related to type 2 diabetes, while HSL gene allele 5, high-density lipoprotein were still negatively related to type 2 diabetes.
CONCLUSION: Our data showed that UCP3 gene allele 6, UCP3 gene allele 7, SBP, abnormity of plasma ApoB and alpha lipoprotein might play a role in the development of type 2 diabetes. HSL gene allele 5, high-density lipoprotein might play some protective role in the development of type 2 diabetes.

L7 ANSWER 92 OF 150 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
DUPLICATE 9
ACCESSION NUMBER: 2001:428374 BIOSIS
DOCUMENT NUMBER: PREV200100428374
TITLE: Is epsilon4 allele of apolipoprotein E associated with more severe end-organ damage in essential hypertension

AUTHOR(S): Yilmaz, Hulya; Isbir, Turgay (1); Agachan, Bedia; Aydin, Makbule

CORPORATE SOURCE: (1) Department of Molecular Medicine, University of Istanbul, Institute for Experimental Medical Research, 34311, Istanbul: tisbir@superonline.com Turkey

SOURCE: Cell Biochemistry and Function, (September, 2001) Vol. 19, No. 3, pp. 191-195. print.
ISSN: 0263-6484.

DOCUMENT TYPE: Article

LANGUAGE: English

SUMMARY LANGUAGE: English

AB The aim of the present study comparing patients with mild to moderate hypertension with controls, was to explore a possible association between hypertension-related target organ damage and evaluation found in the gene encoding apolipoprotein E (apo E) genotype. Detailed medical history was recorded and physical examination was performed for all patients in the study (88 hypertensives, 63 normotensive controls). PCR (Polymerase Chain Reaction), RFLP (Restriction Fragment Length Polymorphism), and agarose gel electrophoresis techniques were used to determine the apo E genotypes. The frequencies of apo epsilon2, apo epsilon3, and apo epsilon4 alleles were 3.97, 88.06, and 9.95%, respectively in the hypertensive group. The frequencies of apo epsilon2, apo epsilon3, and apo epsilon4 alleles were 5.5, 92.0, and 2.38%, respectively in the control group. There were about twice as many individuals in the heterozygote hypertensive group who had apo E3/4 as compared to the control group (7.30 vs. 2.38%) ($p=0.07$). The hypertensive patients who were carriers of the apo epsilon4 had significantly higher organ damage (left ventricular hypertrophy ($p<0.001$). dilated left atrium ($p<0.05$), retinopathy ($p<0.05$)) as compared to those who were not carriers of apo epsilon4. These results showed a trend for the epsilon4 allele to be associated with a higher prevalence of target organ damage in patients with mild to moderate hypertension.

L7 ANSWER 93 OF 150 MEDLINE DUPLICATE 10

ACCESSION NUMBER: 2001346230 MEDLINE

DOCUMENT NUMBER: 21302894 PubMed ID: 11409295

TITLE: Paraoxonase 1 192 Gln/Arg gene polymorphism and cerebrovascular disease: interaction with type 2 diabetes.

AUTHOR: Koch M; Hering S; Barth C; Ehren M; Enderle M D; Pfohl M

CORPORATE SOURCE: Medizinische Universitatsklinik Tubingen, Germany.

SOURCE: EXPERIMENTAL AND CLINICAL ENDOCRINOLOGY AND DIABETES,
(2001) 109 (3) 141-5.

Journal code: 9505926. ISSN: 0947-7349.
PUB. COUNTRY: Germany: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200110
ENTRY DATE: Entered STN: 20011029
Last Updated on STN: 20011029
Entered Medline: 20011025

AB Paraoxonase 1 (PON1) is an HDL-associated enzyme which protects HDL and LDL particles from lipid peroxidation. Its enzymatic serum activity varies 10-40-fold between individuals, and its biallelic gene polymorphism at codon 192 (glutamine-->arginine, Gln/Arg) has been associated with coronary artery disease in diabetic patients. To evaluate the role of this PON1 gene polymorphism in cerebrovascular disease, we determined the PON1 192 genotype in 149 patients with hemodynamically relevant extracranial artery stenosis and in 241 controls. The PON1 192 Gln/Arg genotype was determined using polymerase chain reaction followed by Alw I digestion and polyacrylamide gel electrophoresis. Among all subjects, there was no association between the PON1 192 Gln/Arg genotype and cerebrovascular disease (Odds ratio for Arg/Arg and Gln/Arg vs Gln/Gln 0.99, 95%-CI 0.70-1.39). In contrast, in the subgroup of type 2 diabetic patients the PON1 192 Arg allele conferred about twice the risk of cerebrovascular stenosis compared to those homozygous for the Gln allele (Odds ratio 2.00, 95%-CI 0.92-4.38). Our data indicate that in the general population the PON1 192 Gln/Arg gene polymorphism cannot be regarded as a major risk marker for cerebrovascular disease. The observed interaction with type 2 diabetes, however, is supporting the hypothesis that the effect of the PON1 192 Arg allele on atherosclerosis is modulated by other risk factors like diabetes
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L7 ANSWER 94 OF 150 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
DUPLICATE 11
ACCESSION NUMBER: 2001:418678 BIOSIS
DOCUMENT NUMBER: PREV200100418678
TITLE: Diclofenac induced in vivo nephrotoxicity may involve oxidative stress-mediated massive genomic DNA fragmentation and apoptotic cell death.
AUTHOR(S): Hickey, E. J.; Raje, R. R.; Reid, V. E.; Gross, S. M.; Ray, S. D. (1)
CORPORATE SOURCE: (1) Department of Pharmacology, Toxicology and Medicinal Chemistry, Arnold and Marie Schwartz College of Pharmacy and Health Sciences, Long Island University, Brooklyn, NY,

11201: sray@liu.edu USA
SOURCE: Free Radical Biology & Medicine, (July 15, 2001) Vol. 31, No. 2, pp. 139-152. print.
ISSN: 0891-5849.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Diclofenac (DCLF) is a nonsteroidal anti-inflammatory drug that is widely used for the treatment of osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, and acute muscle pain conditions. Toxic doses of DCLF can cause nephrotoxicity in humans and experimental animals. However, whether this DCLF-induced nephrotoxicity involves apoptotic cell death in addition to necrosis is unknown. The goals of this investigation were to determine whether DCLF-induced nephrotoxicity involves oxidative stress and apoptotic type genomic DNA fragmentation, and if so, whether DCLF-induced oxidative stress and DNA fragmentation cause apoptotic cell death in mouse kidneys. Male ICR mice (CD-1; 25-45 g), fed ad libitum, were administered nephrotoxic doses of DCLF (100, 200, 300 mg/Kg, po) and sacrificed 24 h later. Blood was collected to evaluate renal injury (BUN), lipid peroxidation (MDA: malondialdehyde levels), and superoxide dismutase (SOD) activity (a marker of oxidative stress). Kidney tissues were analyzed both quantitatively and qualitatively to determine the degree and type of DNA damage, and evaluated histopathologically for the presence of apoptotic characteristics in the nucleus of diverse types of kidney cells. Results show that diclofenac is a powerful nephrotoxicant (at 100, 200, and 300 mg/kg: 4.7-, 4.9-, and 5.0-fold increases in BUN compared to the control, respectively) and a strong inducer of oxidative stress (significant increase in MDA levels). Oxidative stress induced by DCLF was also coupled with massive kidney DNA fragmentation (100, 200, and 300 mg/kg: 3-, 8-, and 10-fold increases compared to control, respectively). A dose-dependent increase in MDA levels and SOD activity was also observed, which indicated a link between oxidative stress and nephrotoxicity. Qualitative analysis of DNA fragmentation by gel electrophoresis showed a DNA ladder indicative of Ca2+-Mg2+-endonuclease activation. Histopathological examination of kidney sections revealed numerous apoptotic nuclei across proximal and distal tubular cell linings. Collectively, these data for the first time suggest that DCLF-induced nephrotoxicity may involve production of reactive oxygen species leading to oxidative stress and massive genomic DNA fragmentation, and these two free radical mediated events may ultimately translate into apoptotic cell death of kidney cells in vivo, and reveal a DNA-active role for DCLF.

ACCESSION NUMBER: 2002:377836 BIOSIS

DOCUMENT NUMBER: PREV200200377836
TITLE: Study on base insertion/deletion of CAG-rich region in insulin receptor substrate-1 5'-regulatory sequence.
AUTHOR(S): Huang Jian-jun (1); Song Hui-ping (1)
CORPORATE SOURCE: (1) Department of Biochemistry, Xiangya Medical College, Central South University, Changsha, 410078 China
SOURCE: Hunan Yike Daxue Xuebao, (Apr. 28, 2001) Vol. 26, No. 2, pp. 103-106. print.
ISSN: 1000-5625.
DOCUMENT TYPE: Article
LANGUAGE: Chinese
AB To study the relationship of the polymorphism of the insulin receptor substrate-1 (IRS-1) gene 5'-flanking regulatory sequence and Type 2 diabetes, the IRS-1 gene 5'-flanking regulatory sequence was scanned by PCR-SSCP in 78 healthy control subjects and 76 Type 2 diabetic subjects. Applying PCR-denatured polyacrylamide gel electrophoresis and silver staining, the insertion/deletion polymorphism of the CAG-rich region was analyzed. The genome DNA of the normal and variant subjects was amplified with high-fidelity pfu DNA polymerase. The purified and digested target fragments were then subcloned into the pCAT Basic vector. Each allele was identified according to the mobility by the restrictive endonuclease digestion of the recombinant combined with denatured polyacrylamide gel electrophoresis and silver staining, and finally the constructive plasmids containing different alleles were analyzed by DNA sequencing. Firstly, we found several insertion/deletion variations in the CAG-rich region of IRS-1 gene. Secondly, 7 genotypes and 6 alleles (T1apprxT6) in this site were detected. Moreover, T5 and T6 were only observed in Type 2 diabetic group.

L7 ANSWER 96 OF 150 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:151365 BIOSIS
DOCUMENT NUMBER: PREV200200151365
TITLE: Examination of renin-angiotensin system polymorphisms for association with acute chest syndrome in sickle cell disease.
AUTHOR(S): Keller, Margaret A. (1); Devoto, Marcella; Li, Hui-Hua; Yaakovian, Michael; Schmidt, Linda C.; Ponte, Christine M. (1); Reilly, Anne P.; Sandler, Eric; Ballas, Samir K. (1); Surrey, Saul (1)
CORPORATE SOURCE: (1) Medicine, Jefferson Medical College, Philadelphia, PA USA
SOURCE: Blood, (November 16, 2001) Vol. 98, No. 11 Part 2, pp. 16b. http://www.bloodjournal.org/. print.
Meeting Info.: 43rd Annual Meeting of the American Society

of Hematology, Part 2 Orlando, Florida, USA December 07-11, 2001
ISSN: 0006-4971.
DOCUMENT TYPE: Conference
LANGUAGE: English
AB Acute chest syndrome (ACS) is one of the most serious and life-threatening complications of sickle cell disease (SCD), affecting approximately 10% of patients. We examined the angiotensinogen (AGT) gene microsatellite repeat in a population of African-American SCD patients using chart review to assign patients to ACS or control groups, with ACS defined as acute chest pain associated with the presence of a new pulmonary infiltrate on chest X-ray. The population included both pediatric and adult patients with a mean age of 19 years. The majority of patients were homozygous for Hb S (79% SS, 3.9% Sb0 thalassemia, 11.7% SC, 3.9% Sbeta+ thalassemia and 1.3% SO-Arab with the number of patients co-inheriting beta thalassemia or Hb C not significantly different in cases and controls. No ACS patients two years old or younger were included in this analysis in order to avoid confounding ACS with simple infectious episodes common in this age group. Capillary electrophoresis of fluorescently-tagged AGT PCR products was used to determine AGT microsatellite genotype with select samples being confirmed by DNA sequence analysis. We found that the number of CA dinucleotides located in the 3'-untranslated region of the angiotensinogen gene ranged from 12 to 24. Individuals with the (CA)20 allele were over-represented in the ACS group, with 12 of 41 cases (29%) having at least one CA20 allele compared to only 2 of 36 controls (5.6%). When age and (CA)20 status were examined using multiple logistic regression, the relative risk for carriers of the (CA)20 allele was 5.45 (p=0.020, 95% CI=1.08-27.58). Additionally, studies are underway to examine two coding variants in the angiotensinogen gene and an insertion/deletion polymorphism in the angiotensin converting enzyme (ACE) gene, all of which have been shown to be important in essential hypertension in a variety of ethnic groups. Our preliminary finding of an ACS susceptibility allele in the AGT gene suggests that the renin-angiotensin system plays an important role in ACS, and may represent a new target for therapeutic intervention in SCD.

L7 ANSWER 97 OF 150 MEDLINE DUPLICATE 12
ACCESSION NUMBER: 2001414377 MEDLINE
DOCUMENT NUMBER: 21356634 PubMed ID: 11463752
TITLE: Microsatellite DNA polymorphism of human adrenomedullin gene in normotensive subjects and patients with essential hypertension.
AUTHOR: Ishimitsu T; Hosoya K; Tsukada K; Minami J; Futoh Y; Ono H; Ohrui M; Hino J; Kangawa K; Matsuoka H

CORPORATE SOURCE: Department of Hypertension and Cardiorenal Medicine, Dokkyo
University School of Medicine, Mibu, Tochigi, Japan..
isimitu@dokkyomed.ac.jp
SOURCE: HYPERTENSION, (2001 Jul) 38 (1) 9-12.
Journal code: 7906255. ISSN: 1524-4563.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200108
ENTRY DATE: Entered STN: 20010813
Last Updated on STN: 20010813
Entered Medline: 20010809

AB Adrenomedullin (AM) is a hypotensive peptide widely produced in the cardiovascular organs and tissues. We have cloned and sequenced the genomic DNA encoding the human AM gene and have determined that the gene is located in the short arm of chromosome 11. The 3'-end of the gene is flanked by the microsatellite marker of cytosine adenine (CA) repeats. In this study, we investigated the association between DNA variations in AM gene and the predisposition to hypertension. Genomic DNA was obtained from 272 healthy normotensive subjects (NT) age 57+/-5 years and 266 patients with essential hypertension (EH) age 53+/-11 years. The DNA was subject to PCR using a fluorescence-labeled primer, and the number of CA repeats were determined by poly-acrylamide gel electrophoresis. The averaged blood pressure was 117+/-13/73+/-9 mm Hg in NT and 170+/-23/104+/-12 mm Hg in EH. In Japanese, there existed 4 types of alleles with different CA-repeat numbers: 11, 13, 14, and 19. The frequencies of these alleles were significantly different between NT and EH (chi(2)=9.43, P=0.024). Namely, 13.5% of EH carried the 19-repeat allele, whereas the frequency was 6.2% in NT (chi(2)=7.62, P=0.007). In NT, plasma AM concentrations were not significantly different between the genotypes. In conclusion, microsatellite DNA polymorphism of AM gene may be associated with the genetic predisposition to EH, although the gene expression is not likely to be affected by the genotypes.

L7 ANSWER 98 OF 150 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2000:824511 CAPLUS
DOCUMENT NUMBER: 133:360610
TITLE: Materials and methods relating to disease diagnosis
INVENTOR(S): Franzen, Bo; Hagman, Anders; Ayodele, Alaiya
PATENT ASSIGNEE(S): Karolinska Innovations AB, Swed.
SOURCE: PCT Int. Appl., 45 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000070340	A2	20001123	WO 2000-EP4265	20000511
WO 2000070340	A3	20010208		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

EP 1179175	A2	20020213	EP 2000-931192	20000511

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO

PRIORITY APPLN. INFO.: US 1999-134356P P 19990514
WO 2000-EP4265 W 20000511

AB The invention provides materials and methods relating to disease diagnosis. In particular, the invention provides a method of diagnosing diseases, such as cancers, by comparing specific patterns of gene expression characteristic of the disease at a nucleic acid or protein level. The invention provides novel methods for analyzing the expression profiles characteristic of diseased cells, in order to det. specific diagnostic markers. Such detd. diagnostic markers may be stored on, for example a database, and used in the diagnosis of diseases such as cancer. Ovary tumor samples were analyzed by 2-dimensional gel electrophoresis. Laser densitometer scans were analyzed using PDQUEST software and processed by principal component anal. and by partial least squares discriminant anal. The study suggests that artificial learning strategies can be used for tumor diagnosis.

L7 ANSWER 99 OF 150 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2000:472678 BIOSIS
DOCUMENT NUMBER: PREV200000472678
TITLE: Protein targets of monocrotaline pyrrole in pulmonary artery endothelial cells.
AUTHOR(S): Lame, Michael W.; Jones, A. Daniel; Wilson, Dennis W.; Dunston, Sheryl K.; Segall, H. J. (1)

CORPORATE SOURCE: (1) Dept. of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, One Shields Ave., Davis, CA, 95616 USA
SOURCE: Journal of Biological Chemistry, (September 15, 2000) Vol. 275, No. 37, pp. 29091-29099. print.
ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB A single administration of monocrotaline to rats results in pathologic alterations in the lung and heart similar to human pulmonary hypertension. In order to produce these lesions, monocrotaline is oxidized to monocrotaline pyrrole in the liver followed by hematogenous transport to the lung where it injures pulmonary endothelium. In this study, we determined specific endothelial targets for 14C-monocrotaline pyrrole using two-dimensional gel electrophoresis and autoradiographic detection of protein metabolite adducts. Selective labeling of specific proteins was observed. Labeled proteins were digested with trypsin, and the resulting peptides were analyzed using matrix-assisted laser desorption ionization mass spectrometry. The results were searched against sequence data bases to identify the adducted proteins. Five abundant adducted proteins were identified as galectin-1, protein-disulfide isomerase, probable protein-disulfide isomerase (ER60), beta- or gamma-cytoplasmic actin, and cytoskeletal tropomyosin (TM30-NM). With the exception of actin, the proteins identified in this study have never been identified as potential targets for pyrroles, and the majority of these proteins have either received no or minimal attention as targets for other electrophilic compounds. The known functions of these proteins are discussed in terms of their potential for explaining the pulmonary toxicity of monocrotaline.

L7 ANSWER 100 OF 150 MEDLINE DUPLICATE 13
ACCESSION NUMBER: 2001135314 MEDLINE
DOCUMENT NUMBER: 20529947 PubMed ID: 11079563
TITLE: High-throughput profiling of the mitochondrial proteome using affinity fractionation and automation.
AUTHOR: Lopez M F; Kristal B S; Chernokalskaya E; Lazarev A; Shestopalov A I; Bogdanova A; Robinson M
CORPORATE SOURCE: Proteome Systems, Woburn, MA, USA.. mlopez@genomicsolutions.com
CONTRACT NUMBER: P-01 AG14390 (NIA)
R01 AG15354 (NIA)
SOURCE: ELECTROPHORESIS, (2000 Oct) 21 (16) 3427-40.
Journal code: 8204476. ISSN: 0173-0835.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200103
ENTRY DATE: Entered STN: 20010404
Last Updated on STN: 20010404
Entered Medline: 20010301

AB Recent studies have demonstrated the need for complementing cellular genomic information with specific information on expressed proteins, or proteomics, since the correlation between the two is poor. Typically, proteomic information is gathered by analyzing samples on two-dimensional gels with the subsequent identification of specific proteins of interest by using trypsin digestion and mass spectrometry in a process termed peptide mass fingerprinting. These procedures have, as a rule, been labor-intensive and manual, and therefore of low throughput. The development of automated proteomic technology for processing large numbers of samples simultaneously has made the concept of profiling entire proteomes feasible at last. In this study, we report the initiation of the (eventual) complete profile of the rat mitochondrial proteome by using high-throughput automated equipment in combination with a novel fractionation technique using minispin affinity columns. Using these technologies, approximately one hundred proteins could be identified in several days. In addition, separate profiles of calcium binding proteins, glycoproteins, and hydrophobic or membrane proteins could be generated. Because mitochondrial dysfunction has been implicated in numerous diseases, such as cancer, Alzheimer's disease and diabetes, it is probable that the identification of the majority of mitochondrial proteins will be a beneficial tool for developing drug and diagnostic targets for associated diseases.

L7 ANSWER 120 OF 150 MEDLINE DUPLICATE 22
ACCESSION NUMBER: 1998410782 MEDLINE
DOCUMENT NUMBER: 98410782 PubMed ID: 9740066
TITLE: Effects of renovascular **hypertension** on myocardial protein patterns: analysis by computer-assisted **two**-dimensional gel **electrophoresis**.
AUTHOR: Pleissner K P; Regitz-Zagrosek V; Krudewagen B; Trenkner J; Hocher B; Fleck E
CORPORATE SOURCE: Department of Internal Medicine/Cardiology, Charite, Campus Virchow-Clinic, Humboldt University, Berlin, Germany.. pleiss@dhzb.de
SOURCE: ELECTROPHORESIS, (1998 Aug) 19 (11) 2043-50.
Journal code: 8204476. ISSN: 0173-0835.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199901
ENTRY DATE: Entered STN: 19990128
Last Updated on STN: 19990128
Entered Medline: 19990114

AB Hypertensive heart **disease** caused by renovascular **hypertension** reflects the response of the heart to an increased afterload and neurohormonal stimulation. We hypothesized that in this condition the composition of the myocardial proteins of rats was altered. To identify yet unknown quantitative and qualitative differences in myocardial proteins in renovascular hypertensive heart **disease**, we analyzed protein patterns by computer-assisted **two** -dimensional polyacrylamide large gel **electrophoresis**. Renovascular **hypertension** was induced by placing a silver clip on the left renal artery in 9-week-old rat siblings. Sham-operated animals served as controls. Systolic blood pressure (197 +/- 19 mm Hg) and heart/body weight ratios (0.36 +/- 0.04%) were significantly increased in the hypertensive animals. Twenty protein patterns from the left ventricle of five hypertensive and five control rats were compared. The molecular mass and isoelectric point (pI) of proteins spots ranging from 13 to 100 kDa and from 4.5 to 8.5, respectively, were determined using **marker** proteins. In total, 761 +/- 88 protein spots were resolved in all twenty gels. For the quantitative data analysis a univariate (Mann-Whitney test) as well as a multivariate statistical approach (correspondence analysis) were applied. Only one myocardial protein spot (molecular mass = 41.3 kDa; pI = 6.3) was decreased by more than twofold ($p < 0.05$) in renovascular **hypertension**. The vast majority of spots did not indicate a significant alteration of intensity. Left ventricular hypertrophy in early renovascular **hypertension** induces a form of myocardial hypertrophy that conserves the naturally occurring protein expression pattern.

L7 ANSWER 124 OF 150 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. DUPLICATE 24
ACCESSION NUMBER: 1998:490397 BIOSIS
DOCUMENT NUMBER: PREV199800490397
TITLE: Relationship between the amounts of high molecular mass alkaline phosphatase and beta2-microglobulin in patients with diabetic renal failure.
AUTHOR(S): Tsumura, Mayumi (1); Ueno, Yoshito; Kuwata, Shoji; Kinouchi, Takashi; Morio, Kazuaki; Komoda, Tsugikazu
CORPORATE SOURCE: (1) Dep. Clinical Lab., Teikyo Univ. Sch. Med., Ichihara Hosp., 3426-3 Anesaki, Ichihara 299-0111 Japan
SOURCE: Japanese Journal of Electrophoresis, (Aug., 1998) Vol. 42, No. 3, pp. 169-174.
ISSN: 0031-9082.
DOCUMENT TYPE: Article
LANGUAGE: Japanese

SUMMARY LANGUAGE: Japanese; English

AB We found frequently an atypical alkaline phosphatase (ALP) band which detected at the upper region of the separating gel (atypical ALP-1) by the method of polyacrylamide disc gel (PAG) **electrophoresis** in the patients with certain renal failure. Although most of clinicians have demonstrated the assay of beta-N-acetylglucosaminidase (NAG) and beta2-microglobulin (beta2 MG) as the useful **marker** for advanced renal failure, we then examined the relationship between the levels of beta2MG and the atypical ALP-1 in the patients with chronic renal failures. In particular, we studied the relationship between atypical ALP and beta2-MG in **two** groups of chronic renal failure, one is with **diabetes** mellitus (DM) and the other is without **diabetes** mellitus (non DM). From the present results, higher appearances of the atypical ALP-1 in the serum or urine of the patient with DM nephropathy groups were well accorded to the levels of serum or urinary beta2 MG in the matched patient group. Taken together, the blood or urinary appearance of atypical ALP-1 and the level of beta2MG may be a good tool for the pathological conditions in the patients with diabetic nephropathy.

L7 ANSWER 133 OF 150 MEDLINE DUPLICATE 28
ACCESSION NUMBER: 97037804 MEDLINE
DOCUMENT NUMBER: 97037804 PubMed ID: 8883432
TITLE: The relevance of chondroitin and keratan sulphate **markers** in normal and arthritic synovial fluid.
AUTHOR: Sharif M; Osborne D J; Meadows K; Woodhouse S M; Colvin E M; Shepstone L; Dieppe P A
CORPORATE SOURCE: University of Bristol Rheumatology Unit, Bristol Royal Infirmary.
SOURCE: BRITISH JOURNAL OF RHEUMATOLOGY, (1996 Oct) 35 (10) 951-7. Journal code: 8302415. ISSN: 0263-7103.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199611
ENTRY DATE: Entered STN: 19961219
Last Updated on STN: 19961219
Entered Medline: 19961127

AB This study investigated the synovial fluid concentrations of glycosaminoglycan (GAG), keratan sulphate (KS) epitope 5D4 and chondroitin sulphate (CS) sulphation patterns in healthy volunteers and patients with **osteoarthritis** (OA) and rheumatoid arthritis (RA). Synovial fluids were collected from knee joints of healthy volunteers (n = 24), and patients with OA (n = 28) and RA (n = 29). Concentrations of GAG and the keratan sulphate epitope 5D4 were measured in 15 of the healthy volunteers, and all of the OA and RA synovial fluids. Total GAG was measured using a dye-binding method and 5D4 by an ELISA. The unsaturated CS disaccharides delta C4 and delta C6 were measured by capillary **electrophoresis** in all synovial fluids. The concentrations of GAG, 5D4 and delta C6 in the normal synovial fluid were higher but that of delta C4 lower than those of the **disease** groups. The delta C6:delta C4 ratios correlated with age ($r = -0.437$, $P < 0.001$) and the mean value was lower in females than males (**2**.92 compared with 5.22, $P < 0.001$). After allowing for age and sex, the delta C6:delta C4 ratio in the control group was significantly elevated ($P < 0.001$) compared to both OA and RA. The ratio was also related to proteoglycan **markers** ($r = 0.383$ for 5D4 and $r = 0.357$ for GAG). The finding that 5D4 and delta C6:delta C4 ratios are higher in synovial fluid from healthy volunteers compared to OA and RA suggests that they may be **markers** of the susceptibility of articular cartilage to early damage in arthritis.

L7 ANSWER 136 OF 150 MEDLINE DUPLICATE 29
ACCESSION NUMBER: 95294467 MEDLINE

DOCUMENT NUMBER: 95294467 PubMed ID: 7775869
TITLE: High density lipoprotein subfractions in non-insulin-dependent **diabetes** mellitus and coronary artery **disease**.
AUTHOR: Syvanne M; Ahola M; Lahdenpera S; Kahri J; Kuusi T; Virtanen K S; Taskinen M R
CORPORATE SOURCE: First Department of Medicine, University of Helsinki, Finland.
SOURCE: JOURNAL OF LIPID RESEARCH, (1995 Mar) 36 (3) 573-82. Journal code: 0376606. ISSN: 0022-2275.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199507
ENTRY DATE: Entered STN: 19950720
Last Updated on STN: 19950720
Entered Medline: 19950712

AB High density lipoprotein (HDL) subfractions (2b, 2a, 3a, 3b, and 3c) separated by gradient gel **electrophoresis** (GGE) and defined by Gaussian summation analysis, and the compositions of HDL2 and HDL3, separated by preparative ultracentrifugation, were studied in four groups of men with or without non-insulin-dependent **diabetes** mellitus (NIDDM) and coronary artery **disease** (CAD): group 1 (DM+CAD+, n = 50); group **2** (DM-CAD+, n = 50); group 3 (DM+CAD-, n = 50); and group 4 (DM-CAD-, n = 31). HDL GGE subfraction distributions, available in 125 subjects, were not significantly different among the groups. In contrast, dividing the whole study population into quartiles of serum triglyceride (TG) concentration showed that high TG levels were significantly associated with low HDL2b and high HDL3b concentrations. In a multivariate linear regression model, postheparin plasma hepatic lipase (HL) activity, and fasting serum insulin and TG concentrations were all associated independently and inversely with low HDL2b, but lipoprotein lipase or cholesteryl ester transfer protein activities were not correlated with HDL2b concentrations. Group 1 tended to have the smallest mean particle sizes in the HDL subfractions, significantly ($P < 0.03$, CAD vs. non-CAD) for HDL2b and for HDL2a. These differences were independent of TG, insulin and HL, but lost their significance when adjusted for beta-blocker therapy. Both HDL2 and HDL3 particles in group 1 were significantly depleted of unesterified cholesterol, and their HDL2 was TG-enriched ($P = 0.053$). A high HL activity, hyperinsulinemia and hypertriglyceridemia are independently associated with low levels of HDL2b and generally small HDL particle size. HDL particles in subjects with NIDDM and CAD are small-sized and have a low free cholesterol content. Both these characteristics may be **markers** of impaired reverse cholesterol transport.

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